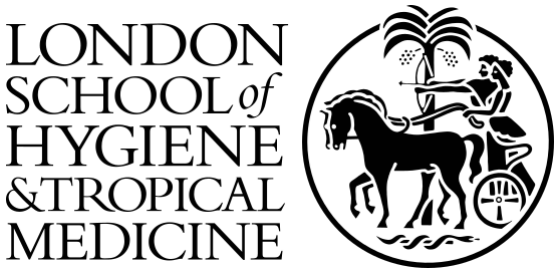

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Assessment of the population-level impact of a high coverage HPV immunisation programme in young females

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Thesis submitted in accordance with the requirements for the degree of

Doctor of Philosophy

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LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE

Funded by Public Health England

Declaration

I, David Mesher, confirm that the work presented in this thesis is my own. Where information had been derived from other sources, I confirm that this has been indicated in the thesis

Signed Date

David Mesher

Abstract

Human papillomavirus (HPV) infection is common in England. Persistent HPV infection can cause cervical and other HPV-related cancers. In clinical trials, HPV vaccination was found to have very high efficacy against HPV infection and early HPV-related disease. The National HPV Immunisation Programme, using HPV16/18 vaccine, was introduced in the UK in September 2008 for females aged up to 18 years old. This thesis aims to evaluate the equity and coverage of HPV vaccination in England and the population-level impact of the vaccination programme on infection and early disease outcomes in young females.

In this thesis, serological surveillance confirmed high coverage of HPV vaccination in the targeted female population. However, surveillance among women at higher risk of HPV infection indicated lower coverage among those born outside of the UK, from more deprived areas or with a previous diagnosis of chlamydia infection.

The same higher-risk population was used to investigate a previous ecological observation of reduced genital warts diagnoses since the vaccination was introduced. I designed and conducted a case-control study to estimate the effectiveness of HPV16/18 vaccination against genital warts (which are largely caused by HPV6/11). This study found no evidence that HPV16/18 vaccination offered cross-protection against warts (adjusted odds ratio (95% CI):1.02 (0.72-1.45)).

My analyses of HPV infection surveillance data within the post-vaccination period (2010-2016) demonstrated substantial declines in prevalence of HPV16/18 infection in 16-18 year olds (8.2% in 2010/2011 compared to 1.6% in 2016) and of HPV31/33/45 (6.5% to 0.6%). This work provides evidence of substantial direct protection against HPV16/18 and some type-specific cross-protection. It also shows

a strong herd protection effect of vaccination. Reassuringly, there was no evidence of other non-vaccine types becoming more common.

The results of this thesis will inform future decisions about changes to the National HPV Immunisation Programme and the UK Cervical Screening Programme.

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First and foremost, I am so grateful to my two supervisors whose combined wisdom and guidance has been invaluable. Sara Thomas has been not only incredibly insightful but also a wonderfully supportive supervisor. She has inspired me to improve over the years of the PhD and I have learnt so much from her. I am often awed by her ability to be focussed and perceptive while multi-tasking on so many projects; I do not know how she does it but I am so glad that she does. Kate Soldan has been an encouraging and extremely motivating supervisor. Her exceptional knowledge of HPV epidemiology and prevention has not only been hugely beneficial throughout this PhD, but also fuelled my own enthusiasm for the subject.

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This PhD would not have been possible without the support of Public Health England; not only financially, but also for providing me with the opportunity to lead on the evaluation of national HPV vaccination in England at such an exciting time for HPV prevention.

I am also grateful for the support of fellow PhD students (past and present); thanks to Katy, Sara, Adamma, Sarika, Meaghan, Koh Jun and Sarah from PHE; Helen, Lizzie, Andreia, Anu, Kate, Emily and Clara from LSHTM; and Alex, Anita and Iva. And to all my friends and family, thanks for listening to my grumbles and not rolling your eyes too much when, repeatedly, I made the excuse; “I’m working on my PhD”. And finally, a big thanks to my husband, Kamran, who has been incredibly supportive and patient, particularly over the last few months of this PhD.

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Abbreviations

AORRP	Adult onset recurrent respiratory papillomatosis
ASC	Adeno-squamous carcinoma
AT	Area team
BASHH	British Association on Sexual Health and HIV
CaSH	Community and sexual health (or contraceptive and sexual health)
CGIN	Cervical glandular intraepithelial neoplasia
CHIS	Child health information system
CHRD	Child Health Reference Department
CI	Confidence interval
CIN	Cervical intraepithelial neoplasia
CPRD	Clinical Practice Research Datalink
CTAD	Chlamydia Testing Activity Dataset
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
EMA	European Medicines Agency
GMC	Geometric mean concentration
GP	General practitioner
GUM	Genitourinary medicine
HC2	Hybrid Capture 2
HIV	Human immunodeficiency virus
HPV	Human papillomavirus
IgG	Immunoglobulin G
IARC	International Agency for Research on Cancer
IMD	Index of multiple deprivation
JCVI	Joint Committee on Vaccination and Immunisation
JORRP	Juvenile onset recurrent respiratory papillomatosis
LA	Local authority
LBC	Liquid-based cytology
LCR	Long control region
LSOA	Lower layer super output area
MSM	Men who have sex with men
NCSP	National Chlamydia Screening Programme
NHAIS	National Health Applications and Infrastructure Services
NHS	National Health Service

OR [aOR]	Odds ratio [adjusted odds ratio]
PAHO	Pan American Health Organization
PCR	Polymerase chain reaction
PCT	Primary Care Trust
PDH	Pyruvate dehydrogenase
PHE	Public Health England
PHOF	Public Health Outcomes Framework
PID	Pelvic inflammatory disease
PPV	Positive predictive value
PR [aPR]	Prevalence ratio [adjusted prevalence ratio]
pRB	Retinoblastoma protein
QALY	Quality-adjusted life year
RCT	Randomised controlled trial
REC	Research ethics committee
RLU/CO	Relative light units/cut-off
RMITT	Restricted modified intention to treat
RRP	Recurrent respiratory papillomatosis
SCC	Squamous cell carcinoma
SE	Standard error
SEU	Sero-epidemiology Unit
SHS	Sexual health service
SIL	Screening and Immunisation Lead
SIT	Screening and Immunisation Team
STI	Sexually transmitted infection
TVC	Total vaccinated cohort
UK	United Kingdom
USA	United States of America
VE	Vaccine effectiveness
VEU	Vaccine Evaluation Unit
VLP	Virus-like particle
VRD	Virus Reference Department
VVS	Vulva-vaginal swab
WHO	World Health Organization

Chapter 1: Introduction

Human papillomavirus (HPV) infection is very common in both men and women in England, particularly among younger age-groups. Although a large proportion of HPV infections clear on their own without manifesting symptoms, HPV-related cancers and genital warts still cause substantial ill health and reduced quality of life in those affected. Since effective vaccines became available in 2007, HPV vaccination programmes have been introduced in many high-income countries. In England, the National HPV Immunisation Programme was introduced in 2008 with the primary aim to reduce the incidence of cervical cancer in women. Vaccination is offered routinely to females in Year Eight of schooling (those aged 12 years on the previous 1st September). Females remain eligible for vaccination up to age 18 years old and, in the first two years of the programme, there was also a catch-up campaign specifically targeting these older females. In Chapter 2 of this PhD, I describe in more detail the epidemiology of HPV infection and related diseases prior to the introduction of HPV vaccination. I also describe the licensed HPV vaccines and give further details about the roll-out of HPV vaccination in the UK and in other parts of the world.

At the time of the introduction of these vaccination programmes, a reduction in the prevalence of the HPV vaccine types was expected to be seen over the following years and many countries established surveillance systems to monitor this. Evidence of these changes has begun to emerge, and can be used to evaluate past decisions and performance and to inform future decisions and implementation. This PhD focuses on the impact at a population level of the National HPV Immunisation Programme in England. After the Background (Chapter 2), the thesis is separated broadly into two parts. In the first part (Chapters 3, 4, 5 and 6) I explore changes in the prevalence of HPV infection. In the second part (Chapters 7 and 8) I study the

serological response to the vaccine HPV types as a biological marker to estimate vaccination coverage. The chapters are summarised briefly below:

Chapter 3 comprises an international systematic review and meta-analysis of changes in non-vaccine HPV types since the introduction of HPV vaccination programmes. Through this work I explored whether reductions in vaccine types were associated with increases in other HPV types.

Chapter 4 provides details of the HPV infection surveillance established in England to monitor and evaluate the National HPV Immunisation Programme. In this chapter, I describe the strengths and limitations of the surveillance data and methods I adopted as part of this PhD to strengthen this surveillance and facilitate accurate interpretation of the results.

Chapter 5 details methods to collect individual HPV vaccination records for a subset of women included in the HPV infection surveillance. I also present results of a validation study to compare vaccination status from different data collection systems.

Chapter 6 presents the results of the HPV infection surveillance described in Chapter 4, in which I investigated changes in the prevalence of HPV infection in young women in England since the introduction of HPV vaccination.

In Chapter 7, I describe the data sources and methods used for two surveillance studies to monitor HPV antibodies in residual sera specimens and methods to ascertain HPV vaccination status using these data. This chapter also includes the methods of a nested case-control study which I designed and conducted as part of this PhD. This study was designed to explore a previous unexpected ecological observation of reductions in the incidence of genital warts associated with HPV16/18 coverage.

In Chapter 8, I report the analyses of data from these two serology surveillance studies to validate HPV vaccination coverage in England and explore variations in subgroups of the populations studied. I also present the results and further discussion of the nested case-control study that was conducted to estimate the effect of vaccination on the incidence of genital warts.

The final chapter, Chapter 9, includes a discussion of the public health importance of these first results showing an impact of HPV vaccination in England. I discuss the strengths and limitations of using surveillance data to evaluate HPV vaccination, and how this thesis addressed some of these limitations. Finally, I discuss the implications of the results and how they inform future public health policies.

Chapter 2: Background

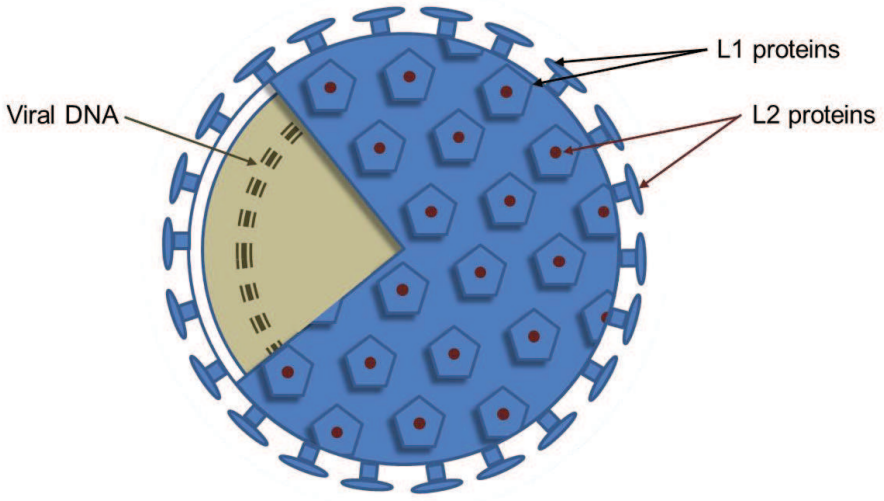
In this chapter I describe the biology and natural history of human papillomavirus (HPV) infection and progression from infection to disease. I review the epidemiology of HPV and related diseases in the UK. I then describe current HPV vaccines and, specifically, the National Immunisation Programme in England. Finally, I explore the rationale and methods proposed to monitor and evaluate this national vaccination programme.

2.1. Biology and natural history of HPV infection

2.1.1 *Human papillomavirus*

The Human papillomavirus (HPV) is a non-enveloped, double-stranded DNA virus which consists of six early genes (E1, E2, E4, E5, E6 and E7), two late genes (L1 and L2), and a non-coding long control region (LCR). The HPV genome is circular with a capsid, around 55-60 nanometres in size, formed of the L1 proteins assembled as 72 star-shaped pentamers (Figure 2.1).

Figure 2.1: Structure of human papillomavirus (HPV)



There are over 100 types (or strains) of HPV which are grouped into species which identify closely related types. These species are further grouped into five genera (Alpha-papillomavirus, Beta-papillomavirus, Gamma-papillomavirus, Mu-papillomavirus and Nu-papillomavirus) (Table 2.1)[1].

Table 2.1: Classification of human papillomaviruses within genera and species

Genus	Species	HPV types
Alpha-papillomavirus	1	32, 42
	2	3, 10, 28, 29, 77, 78, 94
	3	61, c62, 72, 81, 83, 84, c86, c87, c89
	4	2, 27, 57
	5	26, 51, 69, 82
	6	30, 53, 56, 66
	7	18, 39, 45, 59, 68, 70, c85
	8	7, 40, 43, c91
	9	16, 31, 33, 35, 52, 58, 67
	10	6, 11, 13, 44, 55, 74, CCPV, PcPV
	11	34, 73
	12	RhPV1
	13	54
	14	c90
	15	71
Beta-papillomavirus	1	5, 8, 12, 14, 19, 20, 21, 24, 25, 36, 47, 93
	2	9, 15, 17, 22, 23, 37, 28, 80
	3	49, 75, 76
	4	92
	5	96
Gamma-papillomavirus	1	4, 65, 95
	2	50
	3	48
	4	60
	5	88
Mu-papillomavirus	1	1
	2	63
Nu-papillomavirus	-	41

Adapted from de Villiers et al [1]

Since the early 1980s, novel HPV types have been identified using a sequential numbering system. Of the HPV types described, approximately 40 infect the genital tract. These genital HPV types have been classified by the International Agency for Research on Cancer (IARC) into four categories: high-risk, probably high-risk, possibly high-risk and low-risk, according to their association with cervical cancer. There are 13 HPV types which have been classified as high-risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59) or probably high-risk (68) with a further seven types classified as possibly high-risk (26, 53, 66, 67, 70, 73, 82) [2]. Other HPV types, classified as low-risk, are associated with genital warts (types 6 and 11; Section 2.4.3) as well as plantar warts and common and flat warts.

2.1.2 *Transient and persistent HPV infection*

Genital HPV infection is common in both men and women and individuals can be infected with a single HPV type or with multiple types. HPV is predominantly sexually transmitted, although penetrative intercourse is not essential for infection[3]. HPV exclusively infects the epithelium cells of the skin and mucous membranes and is thought to infect the basal epithelial cells through micro-abrasions or other epithelial trauma which expose sections of the basal cells[4]. Genital HPV types are found in the cervix, vulva, vagina, penis, anus and rectum. The genital types can also invade the mucous membranes of the mouth, tongue and throat.

A current HPV infection can be detected using molecular testing (Section 2.2). HPV infection cannot be treated but the majority of men and women infected with HPV will be asymptomatic and the infection will clear on its own. Previous studies have shown that approximately 50% of high-risk HPV infections will clear within 6 months of the initial infection, with 80-90% clearing within 24 months[5-7]. Whilst there is no clear biological definition of what constitutes persistent HPV infection, it has been shown that women with a high-risk HPV infection which does not clear within 6

months are at an increased risk of HPV-related disease, hence this is generally the definition used. Persistent high-risk HPV infection of mucosal epithelium can progress to intraepithelial neoplasia. If left untreated, intraepithelial neoplasia can further progress to high-grade abnormalities and potentially cancer (Section 2.4).

2.1.3 *Immune response to natural HPV infection*

Not all individuals who have a natural HPV infection will seroconvert (i.e. subsequently have detectable antibodies) for that particular HPV type. The life cycle of HPV infection is limited to the epithelium and there is no viraemia, hence the level of HPV in lymphatic channels is very low. Furthermore, as there is no cytolysis or necrosis HPV does not cause inflammation and so HPV infections may not provoke an antibody response. Seroconversion following a natural HPV infection is more common in women than in men. In women, around 50-70% of transient HPV-16 infections will produce antibodies to the HPV16 L1 protein [8-11] albeit often only a weak antibody response. The proportion that seroconverts after a persistent HPV infection is higher; one study conducted in a cohort of 588 women showed around 80% seroconverted after persistent HPV DNA infection with type 16 and 60% with HPV 18 [9]. In men, a smaller proportion seroconverts. A study of 156 men showed that only 7% with a transient HPV-16 infection and around 20% with a persistent HPV-16 infection seroconverted for that HPV type[12].

When antibodies are detected after HPV infection, it can take between 3 and 48 months before this occurs and there is poor correlation between seropositivity and current HPV infection [9, 13]. This suggests that these relatively weak antibody responses following natural infection are not a key factor in the clearance of the infection. Furthermore, it is unclear whether antibody responses elicited from a natural HPV infection are sufficient to protect against future reinfection from the same type[14, 15] or infections with other closely related types[16].

2.2. Testing to detect current HPV infection

Several technologies are available to detect a current HPV infection. Tests to detect high-risk HPV types can be broadly separated by objective into research tests and clinical tests. Tests used for research (and/or surveillance) are generally required to have a low detection threshold in order to determine HPV presence (and/or to identify HPV genotypes) in a particular sample. Clinical tests aim to identify samples with high HPV DNA copy numbers, which have been shown to be associated with progression to high-grade cervical disease (Section 2.4).

In this thesis, I focus on research tests for surveillance to monitor HPV prevalence in the population. There are two main approaches to detecting specific HPV types, which both use polymerase chain reaction (PCR) techniques to amplify HPV DNA present in biological specimens.

(i) Consensus PCR assays (otherwise known as broad spectrum assays) aim to amplify multiple mucosal HPV types by targeting a conserved L1 gene. Such methods for HPV detection in specimens use various different consensus primers with varying sensitivity and specificity. However, in specimens with multiple HPV infections these consensus assays may miss under-represented HPV types (i.e. those with lower HPV DNA copy numbers), as these are masked by types with higher HPV DNA copy numbers [17, 18]; the implications of this are explored further in Section 2.7.3. Following consensus PCR techniques, individual HPV types can be identified by sequencing of the amplified DNA.

(ii) Type-specific PCR assays amplify DNA using a type-specific primer and therefore have a higher sensitivity for individual HPV types, even in the presence of multiple infections. However, these assays are more expensive and laborious, especially if considering testing for many HPV types and,

therefore, are not necessarily a suitable approach for large epidemiological studies or infection surveillance.

2.3. HPV infection prevalence and risk factors

Studies conducted prior to the introduction of HPV vaccination in high-income countries have shown prevalence of high-risk HPV infection of 35-45% in the younger sexually active age-groups (under 25 years of age) and decreasing prevalence with increasing age[19-22]. This age-specific pattern of HPV infection is confirmed with data from seroprevalence surveys, which although not accurately reflecting the proportion of women with a current infection (Section 2.1.3), show exposure to HPV from the age of 14-16 years with an initial peak at around 22-24 years[23]. Data from other countries have shown worldwide variations in these age-specific patterns of HPV prevalence, with some Central and South American countries having a second peak in HPV prevalence at an older age, and other countries having high prevalence across all ages[24].

As genital HPV infection is almost entirely sexually transmitted, risk factors associated with HPV infection of the genital tract tend to be similar to risk factors associated with other sexually transmitted infections. Results from a national survey of sexual attitudes and lifestyles (Natsal-3) conducted among 16-45 year olds in Britain between 2010 and 2012 showed infection with high-risk HPV types in women was associated with younger age, not living with a partner, lower socio-economic status, increased number of sexual partners, attendance at a Genitourinary Medicine (GUM) clinic and/or having a previous sexually transmitted infection (STI) diagnosis, smoking and increased alcohol consumption[25]. Another UK study, conducted among 2,369 sexually active young women attending for chlamydia screening, showed increased HPV prevalence was associated with multiple sexual partners and that those with *Chlamydia trachomatis* infection were more likely to be

infected with a high-risk HPV type[19]; the latter finding was not surprising as the two infections have been shown to have very similar risk factors[26]. High-risk HPV infection has also been shown to be associated with ethnicity. Specifically, in the USA, a national survey of 4,150 women aged 14–59 years which was conducted between 2003 and 2006, showed non-Hispanic black women had a higher prevalence of HPV infection compared to non-Hispanic white women [20] although there was less evidence of this after adjustment with other factors (including number of sexual partners and poverty index) suggesting that variations in HPV infection by ethnicity may be partly explained by differences in sexual behaviour. A national survey of 2,569 women conducted in the UK between 2010 and 2012, showed high-risk HPV prevalence was lower in women of Asian ethnicity[25].

2.4. HPV-related disease

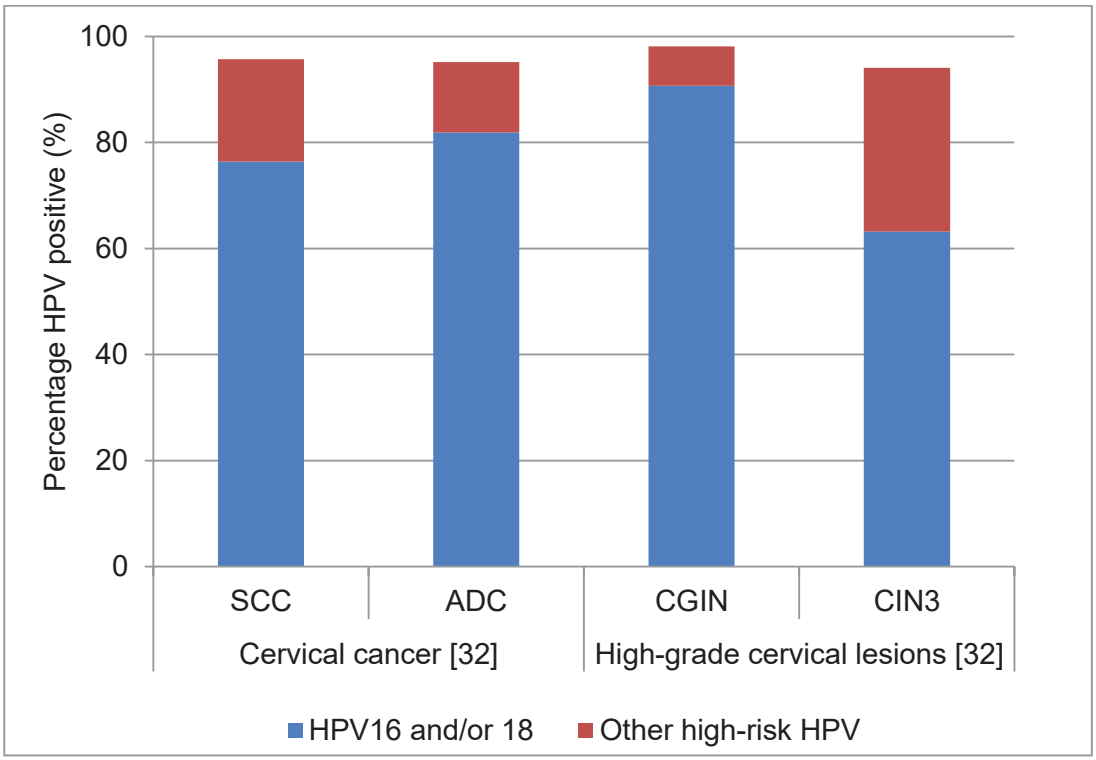
2.4.1 *Cervical cancer*

Persistent infection with one of the high-risk HPV types is a necessary, but not sufficient, cause of cervical cancer [27]. In the cervix, following infection of the basal epithelium cells, there is expression of the early HPV genes (E1, E2, E4, E5, E6 and E7). As the virus replicates and moves to the surface layer of the epithelium the late genes L1 and L2 are expressed. This causes the production of virions which are shed internally at the skin surface. Progression to cancer is associated with integration of the HPV genome into the host chromosome which causes disruption of E2 and leads to overexpression of E6 and E7 oncogenes[28], which in turn inactivates the host's anti-oncogenes p53 and retinoblastoma protein (pRB)[29, 30].

Progression from a persistent HPV infection to cervical cancer takes many years with gradual progression from pre-cancerous low-grade lesions to high-grade lesions and eventually to cervical cancer. The median time from high-grade lesion to cancer is estimated to be over 20 years[31] although not all pre-cancerous lesions progress; some regress without treatment. A large proportion of HPV infections

found in cervical cancer are from one of two species of HPV types; α7 and α 9 (α7 includes HPV18, HPV39, HPV45, HPV59 and HPV 68; α9 includes HPV16, HPV31, HPV33, HPV35, HPV52 and HPV58) (Section 2.1.1). HPV16 and 18 are associated with at least 70-80% of all cervical cancers[32, 33], 50-65% of all high-grade cervical lesions[32, 34] (Figure 2.2) and 25-35% of low-grade cervical lesions[35].

Figure 2.2: HPV prevalence among cervical cancers and high-grade cervical lesions, in England (cases collected between 2000[1] and 2008[32])



SCC = Squamous cell carcinoma; ADC = Adeno and adeno-squamous carcinoma; CGIN = Cervical glandular intraepithelial neoplasia; CIN; cervical intraepithelial neoplasia

1: A small proportion (4%) of cervical cancer cases were collected between 1986 and 2000

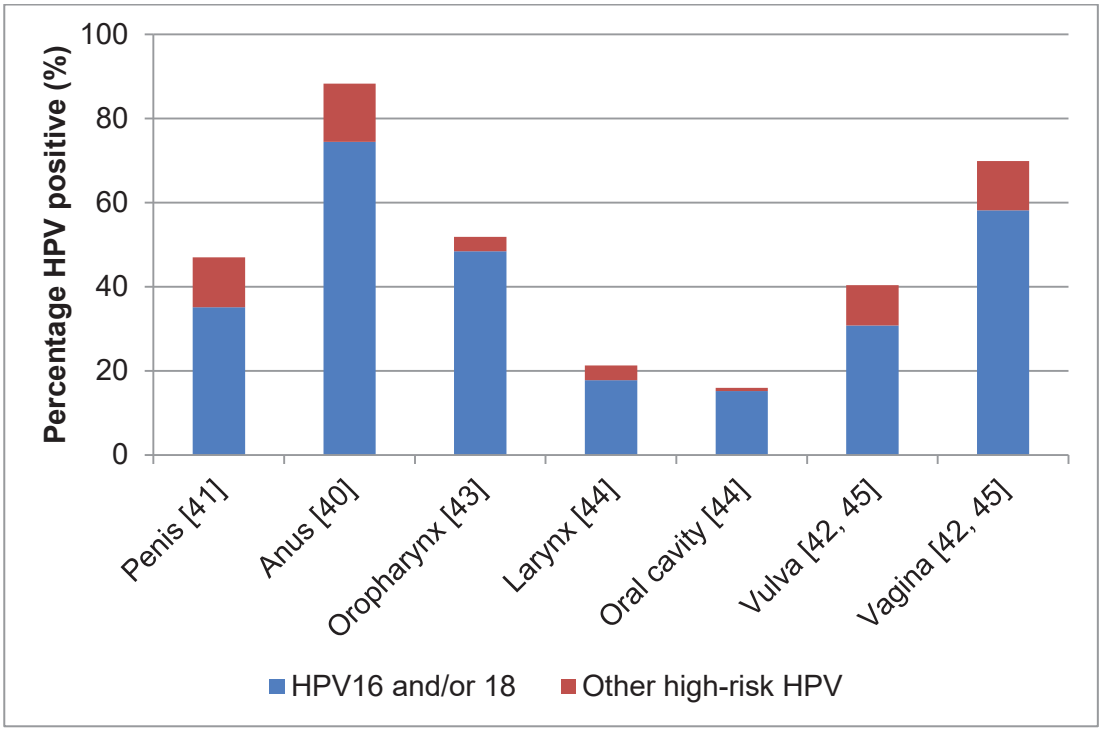
Worldwide, cervical cancer is the 4th most common cancer among women with an estimated 528,000 new cases of invasive cervical cancer and 266,000 deaths in 2012[36]. In the UK, the incidence of cervical cancer is lower and is the 13th most common cancer among women, with 2,517 cases in 2015 (9.2 cervical cancers per 100,000 women) and a peak incidence around 25-29 years of age[37] . These data

represent cervical cancer incidence prior to any effect of HPV vaccination. The lower incidence in the UK and some other high income countries is largely due to effective cervical screening (Section 2.5). The introduction of HPV vaccination programmes, largely in adolescent women, is expected to reduce the incidence of cervical cancer even further as vaccinated females reach the ages at which cervical cancer could be diagnosed.

2.4.2 *Other HPV-related cancers*

HPV has also been shown to be associated with, although not a necessary cause of, cancers at other sites and it is estimated that around 643,000 (~5%) of all cancers worldwide in 2012 were associated with HPV (530,000 from cervical cancer, 113,000 other HPV-related cancers)[38] . These cancers include cancer of the vulva, vagina, penis, anus and some sites of the upper aerodigestive tract[38-45]. The population attributable fractions associated with HPV at these sites are shown in Figure 2.3. Cancers of the vulva, vagina and penis are less common than cervical cancer with 1,081, 197 and 519 cases diagnosed respectively in the UK in 2015, with a much smaller proportion of these cancers attributable to HPV[37]. Cancer of the anus is more common but still has a lower incidence than cervical cervical cancer with 853 cases diagnosed in females and 403 cases diagnosed in males in the UK in 2015[37]. However, there has been a 130% increase in the incidence of anal cancer reported since the mid-1970s[46]. The incidence of anal cancer is disproportionately high in men who have sex with men (MSM) with an estimated annual incidence of around 5.1 per 100,000 for HIV-negative MSM[47] and 45.9 per 100,000 for HIV positive MSM[47]. Finally, data have shown that the incidence of cancers of the aerodigestive tract (including oral cavity, oropharynx, tonsil and larynx) has also been increasing up to 2015[37]. It has been suggested that the cancers which are HPV-related (between 20-30%[44]) are largely responsible for this increase[48, 49].

Figure 2.3: Proportion of cancers which are related to high-risk HPV infection, by cancer site



Penis: Global estimates from systematic review of articles published between 1986 and 2008[41];
Anus: cases diagnosed in Europe between 1986 and 2011[40];
Oropharynx (includes cancer of oropharynx and tonsil): cases diagnosed in the UK between 2002 and 2011[43];
Larynx (includes cancers of the larynx and hypopharynx): European estimates from systematic review of articles published up to 2004[44];
Oral cavity (includes cancer of tongue, gum, floor of mouth, and palate): European estimates from systematic review of articles published up to 2004[44];
Vulva: Proportion attributable to HPV from global estimates from systematic review of articles published between 1986 and 2008[42] / Proportion of HPV positive attributable to HPV16/18 from a different systematic review of articles published up to 2007[45];
Vagina: Proportion attributable to HPV from global estimates from systematic review of articles published between 1986 and 2008[42] / Proportion of HPV positive attributable to HPV16/18 from a different systematic review of articles published up to 2007[45]

2.4.3. Genital warts and recurrent respiratory papillomatosis

Two low-risk HPV types, types 6 and 11, cause the vast majority of genital warts. Whilst genital warts are not usually associated with severe morbidity the demand on health services and the loss of patients' quality of life is considerable. In England, there were 35,374 and 27,342 new cases of genital warts diagnosed in 2016 in males and females respectively[50]. One study of genital warts seen in sexual health clinics (otherwise known as GUM clinics) estimated a quality-adjusted life year (QALY) loss equivalent to 6.6 days of healthy life lost per episode of genital warts, with an average cost of £94 per episode[51].

HPV6 and 11 also cause the rarer but more serious condition of recurrent respiratory papillomatosis (RRP), characterised by warty growths in the respiratory tract which can lead to breathing difficulties and chronic coughing. Treatment for moderate or severe disease has historically been with surgery to debulk the warty growths although more recently the use of intralesional cidofovir has been a major advance in non-surgical treatment of RRP[52]. A cross-sectional study conducted in the UK in 2015 estimated the prevalence of RRP requiring management in secondary or tertiary health sector to be approximately 1.4 per 100,000 population[53]. RRP has a bimodal age distribution. Juvenile onset RRP (JORRP) is usually diagnosed in children under 5 years old and is thought to be a caused by vertical transmission of HPV. Adult onset RRP (AORRP) is less common and less severe. AORRP is usually diagnosed in those aged 30-40 years old and is more common in men than women.

2.5. Secondary prevention: Cervical screening

Cervical screening programmes have been adopted in many countries, and those that have introduced a cervical screening programme which is adequately organised (e.g. that includes training for cervical sample takers, quality assurance of the programme, call/recall systems to invite women at specified time intervals rather

than opportunistic testing) have seen dramatic reductions in the incidence and mortality associated with cervical abnormalities and cancer[54-56]. In high-income countries, screening programmes have almost exclusively relied on use of the Papanicolaou smear test (Pap smear). Subsequent advances have seen the introduction of liquid-based cytology (LBC) across many screening programmes which involve the collected cells being placed in a preservative rather than on a slide. Both Pap smears and LBC aim to detect nuclear abnormalities in the cells of the cervix. Even more recently, the use of HPV testing as the primary test in the cervical screening programme has been piloted at sites across England and recommended to be fully implemented by 2019. HPV testing for high-risk HPV types has been shown to be more sensitive than cytology to detect high-grade cervical abnormalities, although it is considerably less specific, especially in younger women[57, 58].

The National Health Service (NHS) Cervical Screening Programme was established in England in 1964 with a call/recall system established in 1988 which allowed District Health Authorities to invite all eligible women for screening. Women aged 20-49 were invited for screening at 3-yearly intervals and those ages 50-64 at 5-yearly intervals. From 2003, the age women were invited for their first cervical screen in England was raised from 20 to 25 years. The introduction of HPV testing could allow the interval between screens to be extended due to the high long-term negative predictive value of the test[59].

2.6. Primary prevention: HPV vaccination

2.6.1. HPV vaccines

There are currently three prophylactic HPV vaccines which have been licensed by the European Medicines Agency (EMA) for use in Europe; Cervarix® (GlaxoSmithKlein; GSK) is a bivalent vaccine containing the two high-risk HPV types 16 and 18; Gardasil® (MSD Merck) is a quadrivalent vaccine which contains

these two high-risk types and additionally against the low-risk types 6 and 11; and Gardasil® 9 (MSD Merck) which is a nonavalent vaccine which contains the same types as the quadrivalent vaccine as well as an additional 5 high-risk types (31, 33, 45, 52 and 58).

These vaccines work in a similar way; the L1 proteins which form the HPV capsid are re-produced using insect or yeast cells. These synthesised proteins then form L1 pentamers which self-assemble into virus-like particles (VLPs). These VLPs are not infectious or oncogenic as they contain no viral DNA but when inoculated intramuscularly, elicit a type-specific antibody response which is far greater than that elicited by natural infection[60] and is sufficient to protect against future infection of these HPV types (Section 2.6.2). As these HPV vaccines are relatively new, the duration of the immune response following vaccination is as yet unknown. However, data from clinical trials show that HPV antibodies remain for at least 10 years after vaccination with the bivalent vaccine[61] and 8.5 years after vaccination with the quadrivalent vaccine[62] with high antibody levels suggesting that these vaccines will generate a longer term immune response. The duration of protection for Gardasil 9 is not yet known although data from clinical trials have demonstrated non-inferior immune responses for the HPV types included in the quadrivalent vaccine[63]. The reason for the enhanced immunogenicity after vaccination compared to natural infection is thought to be due to the adjuvants used, the optimal dosing schedules and the fact that the vaccines are delivered intramuscularly so VLPs can access the lymphatic system and activate B cells and dendritic cells.

2.6.2. Efficacy of HPV vaccines

For both the bivalent and quadrivalent vaccine, phase III clinical trials have demonstrated 100% vaccine efficacy against HPV16- and HPV18-related pre-cancerous lesions when administered to HPV-naïve women (i.e. women who were HPV DNA negative and seronegative at baseline) compared to a placebo [60, 64].

The quadrivalent vaccine has also been shown to provide almost 100% protection against HPV6- and HPV11-associated anogenital warts in HPV naïve males and females (with vaccine efficacy of 91.6% against HPV-6 related lesions and 100% against HPV-11 related lesions)[65]. The nonavalent vaccine additionally has a high vaccine efficacy (96.7% in the per protocol analysis) against HPV-31, 33, 45, 52, 58 related cervical, vulvar and vaginal disease[63].

For all three vaccines, nearly 100% of women will seroconvert after vaccination with high HPV antibody titres[63, 66, 67]. However, the vaccine efficacy against disease depends on current and previous exposure to HPV infection. HPV vaccination of women with a current HPV DNA infection has not been shown to have any effect on clearance of the existing infection or reducing progression to disease progression. It is not clear whether vaccination has an impact on re-infection with the same HPV type among those who were infected at the time of receiving the vaccine. There is also some evidence of a slightly reduced protective effect of HPV vaccination against pre-cancerous abnormalities among young women currently not infected with HPV but with serological evidence of previous exposure[68, 69]. Whilst any serological correlates of protection for HPV have yet to be determined, it is thought that high concentrations of neutralising antibodies to HPV which are elicited by HPV vaccination play an important role in protecting against future HPV infection. Serological assays to monitor other immune responses (e.g. IgG antibodies or binding antibodies to proteins) following vaccination can be used as a proxy for assays measuring HPV-neutralising antibodies and have the benefit of being less complex than the neutralising assays and allow high throughput testing.

Clinical trials with disease endpoints have largely been conducted in young adult women receiving a 3-dose schedule of vaccine. Clinical endpoints for women vaccinated at younger ages will require longer follow-up, given the time between HPV infection and presentation with cervical abnormalities. However, other clinical

trials have demonstrated non-inferior immune responses (compared to 3-doses in older women) for younger women aged 9-15 years old at vaccination receiving 3 vaccine doses or 2 vaccine doses (given at least 6 months apart). These immunogenicity studies ("immunological bridging studies") have the benefit of shorter follow-up as they consider the endpoint of immune responses following vaccination rather than disease outcomes which may not occur for years following vaccination. Such studies[70, 71] have been the basis for extending the European licence of HPV vaccination to a 2-dose schedule for females aged under the age of 15 years old at the time of the first dose (with 3-doses still recommended for older women) and have led to similar recommendations by the World Health Organisation (WHO)[70, 72].

2.6.3. Cross-protection against closely related HPV types

Phase III studies have also demonstrated evidence that both the bivalent and quadrivalent vaccine provide a level of cross-protection against some closely-related HPV types (Table 2.1) not included in the vaccines.

Evidence for cross-protection from the clinical trials is summarised in Table 2.2. To summarise, among HPV-naïve women at vaccination, vaccine efficacy for the bivalent vaccine against 6-month persistent infection with HPV types 31, 33 and 45 was 77.1% (95% CI: 67.2-84.4), 43.1% (95% CI: 19.3-60.2) and 79.0% (95% CI: 61.3-89.4) respectively[73]. Furthermore, a post-hoc analysis of one trial showed an unexpected moderate cross-protective efficacy against HPV6 [74] which is discussed and explored in more detail in the case-control study reported in Section 8.4. For the quadrivalent vaccine, there is less evidence of cross-protection from the published clinical trials, with only HPV type 31 showing an appreciable vaccine efficacy of 46.2% (95% CI: 15.3-66.4)[75]. It has been suggested that the reason for the difference in cross-protection between the bivalent and quadrivalent vaccine could be due to the different adjuvants used for the two vaccines[76].

Table 2.2: Evidence from Phase III studies for cross-protection against persistent infection (at 6- or 12-months) of non-vaccine HPV types

HPV type		HPV vaccine	Study	Vaccine efficacy (95% CI)
High-risk HPV types	HPV31	Bivalent	PATRICIA[1]	77.1% (67.2-84.4)
		Quadrivalent	FUTURE I/II[2]	46.2% (15.3-66.4)
	HPV33	Bivalent	PATRICIA[1]	43.1% (19.3-60.2)
		Quadrivalent	FUTURE I/II[2]	28.7% (-45.1-65.8)
	HPV45	Bivalent	PATRICIA[1]	79.0% (61.3-89.4)
		Quadrivalent	FUTURE I/II[2]	7.8% (-67.0-49.3)
	HPV51	Bivalent	PATRICIA[1]	25.5% (12.0-37.0)
		Quadrivalent	FUTURE I/II[2]	Not available
	HPV52	Bivalent	PATRICIA[1]	18.9% (3.2-32.2)
		Quadrivalent	FUTURE I/II[2]	18.4% (-20.6-45.0)
	HPV58	Bivalent	PATRICIA[1]	-6.2% (-44.0-21.6)
		Quadrivalent	FUTURE I/II[2]	5.5% (-54.3-42.2)
Low-risk HPV types	HPV6	Bivalent	PATRICIA[3]	34.9% (11.3-51.8)
		Quadrivalent		Not applicable
	HPV11	Bivalent	PATRICIA[3]	30.3% (-45.0-67.5)
		Quadrivalent		Not applicable

1: Total vaccinated cohort naïve (TVC-naïve) analysis included 5427 vaccinated vs 5399 control subjects. Participants were women aged 15-25 years.[73]

2: Restricted modified intention to treat (RMITT) analysis included 1036 vaccinated vs 1032 control subjects. Participants were women aged 16-26 years.[75]

3: TVC-naïve post-hoc analysis included 5259 vaccinated vs 5249 control subjects. Participants were women aged 15-25 years.[74]

Both TVC naïve and RMITT analyses included women without evidence of high-risk HPV infection at baseline

2.6.4. HPV vaccination programmes

A survey at the end of 2014 found that since 2006, 64 countries had implemented a national HPV vaccination programme, four countries had introduced a programme sub-nationally and 12 overseas territories had implemented vaccination[77]. In England (as throughout the rest of the UK), the National HPV Immunisation Programme was introduced in September 2008 with a 3-dose schedule for all girls aged 12-13 years old, using the bivalent vaccine Cervarix®. A catch-up campaign for all girls up to age 18 was offered in the first two years of the programme (as outlined in Chapter 1). Routine vaccination of 12-13 year olds has continued, although from September 2012 there was a change in the vaccine offered from Cervarix® to the quadrivalent vaccine Gardasil®. Following emergence of immunological evidence (Section 2.6.2) it was reconsidered that from September 2014, routinely vaccinated girls need receive only two doses of the vaccine; the second dose being given at least 6- and at most 24-months following the first dose.

In England, vaccination for the routine programme has almost entirely been provided at schools. However, for the older girls, both school-based and GP-based vaccination was offered. There has been consistently high vaccination coverage for the routine cohorts, with administration data reporting national coverage above 85% for one dose and above 80% for all three doses since the introduction of the programme[78-81]. Among the catch-up cohorts, coverage has been lower, with 3-dose coverage ranging from 39% to 76% and a larger proportion of women receiving partial vaccination (between 2.3% and 6.8% receiving only 1-dose and between 3.9% and 11.9% only 2-doses)[78-80]. Ecological analysis of HPV vaccination coverage suggests that area-level deprivation has little impact on vaccination coverage for school-based vaccination delivery, although there was some evidence of inequality of uptake among the girls vaccinated at an older age, with lower recorded vaccination coverage in areas with higher deprivation[82].

2.7. Surveillance to monitor HPV in England

2.7.1. The role of Public Health England and UK health departments

The Joint Committee on Vaccination and Immunisation (JCVI) is an independent Departmental committee of experts which advises the UK health departments on vaccination for the prevention of infections and/or disease. Following a recommendation from JCVI, it is the obligation of the Secretary of State to ensure that such a vaccination programme is implemented. The Department of Health and Social Care is responsible for determining the Immunisation policy in England. However, much of the implementational and operational aspects of national immunisation programmes are delegated to Public Health England (PHE) which is an executive agency of the Department of Health and Social Care.

An important requirement of implementing national immunisation programmes is conducting surveillance to monitor the coverage and impact of vaccination on infection and/or disease. Monitoring vaccination in an unselected population (and under public health conditions of vaccine storage, handling, and administration) provides important information that is not available from clinical trials. Such surveillance identifies whether a programme is working well and potentially informs any changes to existing programmes. It is the responsibility of PHE to carry out surveillance to evaluate and inform national immunisation programmes and to report back to JCVI.

2.7.2. Monitoring the coverage of the HPV vaccination programme

National monitoring of vaccination coverage data provides the first evidence of the success of the programme to deliver HPV vaccine to the targeted cohorts and can identify subgroups of the population with lower coverage.

Individual-level vaccination status should be recorded on the local Child Health Information System (CHIS) and in GP clinical records of eligible girls. In addition,

HPV vaccination details should be uploaded on to the National Health Applications and Infrastructure Services (NHAIS) system (otherwise known as Open Exeter) so that these records can be linked to cervical screening records once women become eligible for the NHS Cervical Screening programme. Full details of how these data are recorded are provided in Section 5.1.1.

In addition, annual reports of area-level HPV vaccination coverage are produced by the Immunisation Department at PHE. These data are collected via the ImmForm website, using a manual online data submission by NHS England Area Teams (ATs). ATs are notified of their individual denominators (i.e. the Area's 'responsible population' which is derived from the relevant school roll, or from the Child Health Information system for cohorts/areas not using a school-based delivery) prior to the start of the academic year to provide the opportunity to amend these where appropriate. Numerator data on HPV vaccine doses given in schools or GP surgeries are then collated by ATs and entered onto ImmForm. Data provided are aggregate counts by year of age and area. Coverage by other characteristics (e.g. ethnicity and deprivation) is not available from this national data collection. I summarise the results of studies which have investigated variations in HPV vaccination uptake and completion (Table 7.1, Section 7.2.2). In Chapter 7, I also describe work I have done in this PhD to monitor and validate HPV vaccine coverage data using biological markers of vaccination. Vaccine-induced antibodies are elicited in almost all vaccinated women and these are substantially higher than those following natural infection (Section 2.6.1). Therefore, dual seropositivity for both HPV16 and HPV18 with high antibody concentrations strongly suggests a vaccine-induced response. In this thesis, I present two seroprevalence surveys, conducted among:

(i) Young women (aged 15 to 19 years old) undergoing routine microbiological or biochemical blood tests through participating laboratories across

England. A total of 3,772 residual serum specimens were collected between 2010 and 2013.

(ii) Young women (aged 16 to 20 years old) attending sexual health clinics in England who are likely to be at an increased risk of high-risk HPV infection. These residual serum specimens were linked to data on ethnicity, country of birth, other STI diagnoses and area-level deprivation before being anonymised prior to HPV antibody testing. A total of 3,959 samples were collected and tested between 2011 and 2015.

These data have three important functions. Firstly, they allow validation of vaccine coverage estimated by Public Health England using ImmForm collection. Secondly, they help identification of population subgroups with lower vaccination coverage. Finally, they inform vigilance for changes in immunogenicity over time following vaccination (i.e. antibody waning). Further details of both serological surveillance studies are provided in Chapters 7 and 8.

2.7.3. Evaluation of the HPV vaccination programme

One of the first measurable effects of a national HPV vaccination programme is on HPV infections in young women. Based on the data from clinical trials, it is anticipated that, following the introduction of the programme there will be:

(i) a large reduction in the population-level prevalence of HPV16 and HPV18 infections, and a smaller reduction in the prevalence of closely-related HPV types against which the vaccines were shown to have some cross-protection;

(ii) a lower prevalence of the vaccine and cross-protective HPV types among vaccinated women compared to unvaccinated women (i.e. evidence of vaccine effectiveness against these HPV types);

(iii) a lower prevalence of vaccine HPV types in unvaccinated women compared to women prior to the introduction of HPV vaccination, due to the herd protection effect (i.e. with less HPV infections in the population, there will be less opportunity to infect unvaccinated women);

(iv) no change in the prevalence of other non-vaccine HPV types.

Regarding the last point, whilst it is not expected that there will be a change in the other non-vaccine HPV types, there are two possible reasons to observe increases in the prevalence of non-vaccine HPV types as a result of HPV vaccination. The first is the concern that reductions in the vaccine-related HPV types could lead to other, less common, HPV types filling their ecological niche and becoming more common; this is known as type-replacement. Although there was no evidence of HPV type competition from natural history studies conducted prior to the introduction of HPV vaccination, pneumococcal serotype replacement was seen following 7-valent pneumococcal vaccination in England and Wales[83]. Therefore, the prevalence of other HPV types is being monitored to remain vigilant for any potential increases in non-vaccine HPV types. The second possible cause of an apparent increase in these types is an unmasking effect, an entirely artificial increase which is an artefact of the HPV tests used (as alluded to in Section 2.2). In other words, it may appear that there is an increase in non-vaccine HPV types due to the higher sensitivity of broad spectrum assays to detect non-vaccine HPV types in a post-vaccination population with less HPV16 and HPV18. Quantification of any potential type-replacement is complicated by this unmasking effect. I explore this further in Chapter 3 and Section 4.1.

At the start of this PhD, there was no routine testing of young women for HPV in clinical practice as this is largely an asymptomatic, transient infection with no recommended treatment. In England, a survey was established by PHE in 2008 to determine type-specific HPV DNA prevalence among sexually active 16-24 year old

women[19]. This made use of residual samples from women undergoing chlamydia screening. In this PhD, I compare pre-vaccination findings with type-specific HPV prevalence in 2010 to 2016 following the introduction of HPV vaccination in England. This surveillance, although not population-based, offers a large source of suitable samples from young sexually active women. This PhD includes results of HPV testing of 15,463 samples collected following the introduction of HPV vaccination (for comparison with 2,369 samples which were collected and tested prior to the introduction of HPV vaccination). Further details of this surveillance, including consideration of type-replacement and potential unmasking effects, are provided in Chapters 4, 5 and 6.

Another effect of HPV vaccination that I consider is the impact on early disease outcomes, including genital warts. An ecological analysis conducted prior to this PhD suggested an unexpected moderately protective effect of the bivalent vaccine against genital warts in England[84]. In this PhD, I conducted further analyses, using data from an epidemiological study I designed to investigate this association (Chapter 7). The impact of HPV vaccination on other disease related to high-risk HPV types (i.e. HPV-related pre-cancer and cancer) will be established at PHE at a future date. It will take longer to see such an effect, given the delay between infection and disease onset, hence this falls outside the scope of this PhD.

2.8. PhD rationale and objectives

2.8.1. Rationale

Following the introduction of a vaccination programme, measuring the impact on infection and disease is an effective way to monitor progress and to inform changes and/or advocate continuation of the programme (as described in Section 2.7.3). National surveillance to monitor HPV vaccination programmes in England and elsewhere often compares the changing epidemiology of HPV infection and disease over time using repeat cross-sectional studies. Such surveillance studies provide

useful estimates for the population-level impact of HPV vaccination. However, this approach is subject to issues with continuity of patient selection (e.g. changing service provision affecting patient attendances at certain clinic types) and changing HPV detection methods over time, hence interpretation of changes are complicated. In this thesis, I describe my role in the design, management and analysis of several surveillance activities established to monitor different aspects of the HPV vaccination programme in England. As a further objective of this PhD, I aimed to address some of the issues with interpretation of national surveillance data in two ways. Firstly, I developed and applied novel techniques to enable accurate interpretation of changes in type-specific HPV infection and HPV seroprevalence data. Secondly, I collected additional data and designed and conducted additional epidemiological studies to calculate directly the effects of HPV vaccination on HPV infection and early disease outcomes. Using these methods, I assessed throughout this PhD the early impact of HPV16/18 vaccination on the epidemiology of HPV infection in vaccinated and unvaccinated individuals in England. These analyses will inform further use of HPV vaccines and potential changes to the national cervical screening in the UK and in other countries with high vaccination coverage in young females. In addition, these data could inform introduction of vaccination in countries yet to implement a national programme.

2.8.2. PhD research questions

Type-specific HPV infection and HPV seroprevalence data from PHE's HPV Surveillance programme have been analysed to address the following questions in the first eight years of the National HPV Immunisation Programme:

Research question 1: What is the effect of national HPV vaccination with the bivalent vaccine on infection with HPV16 and/or HPV18? This includes estimation of the population-level impact of the vaccination programme among young women as

well as direct calculation of the vaccine effectiveness against HPV16 and/or 18 infection.

Research question 2: What is the effect of national HPV vaccination with the bivalent vaccine on infection with other high-risk HPV types? This research question comprises investigation of reductions in the prevalence of closely related HPV types (i.e. cross protection) and potential changes in other non-vaccine HPV types (either decreases due to cross-protection or increases due to potential type-replacement). For non-vaccine types, I look at the population-level impact in young women and vaccine effectiveness, similar to research question 1.

Research question 3: What is the evidence for a herd protection effect of the HPV vaccination programme on women known to be unvaccinated?

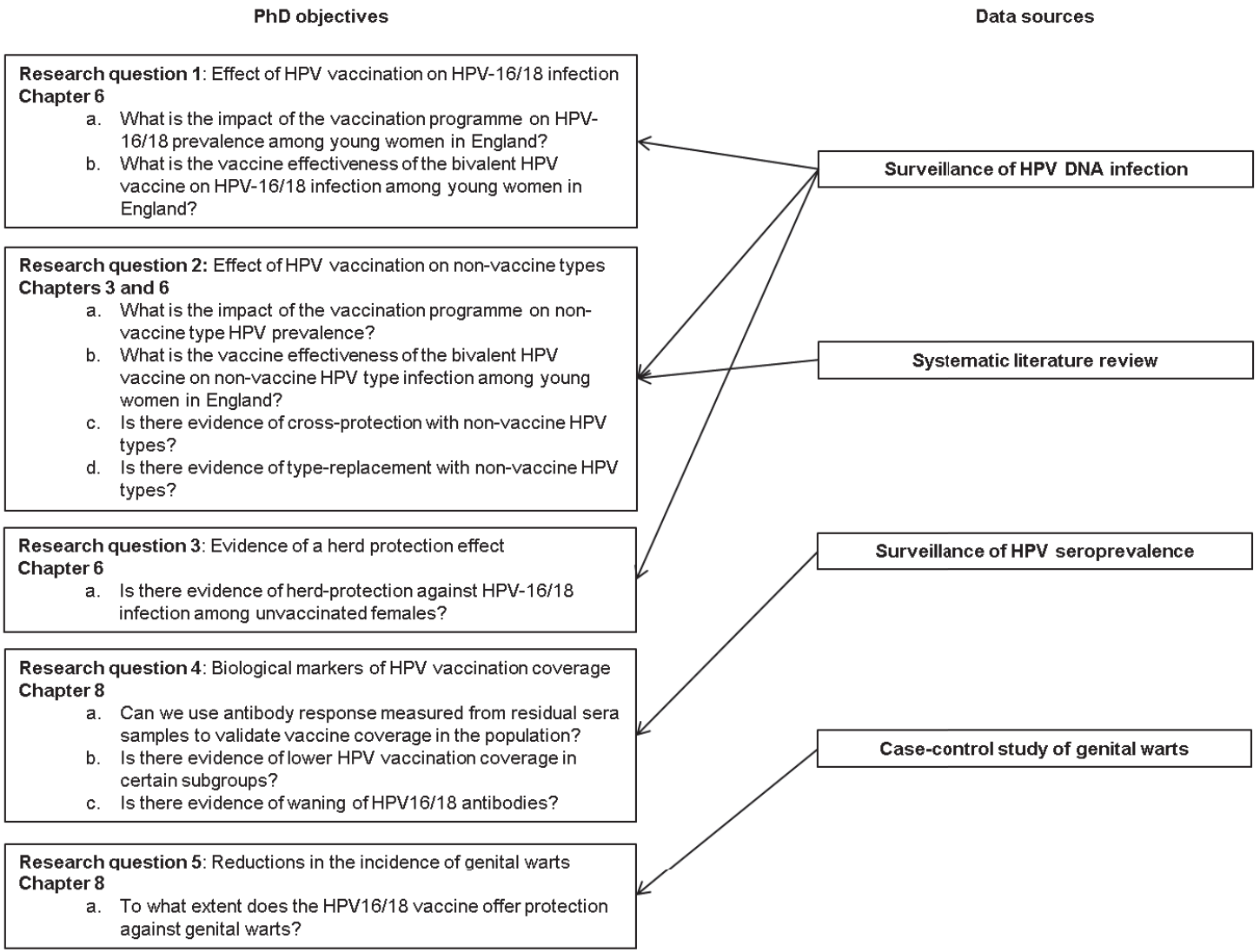
Research question 4: What is the prevalence of biological markers of HPV vaccination coverage in vaccinated populations? To answer this research question, I initially investigate whether monitoring the antibody response to HPV vaccine types is a suitable marker to monitor HPV vaccination coverage. I then use this approach to estimate HPV vaccination coverage among vaccinated women and to explore variations in coverage in different population subgroups. Finally, I investigate whether there is evidence of HPV antibodies waning over time since vaccine introduction.

In addition, I designed and conducted an epidemiological study (case-control) to address the following question:

Research question 5: To what extent does the bivalent HPV vaccine offer protection against genital warts?

Further detail about the data sources used for each question, and in which Chapters of the thesis each question is addressed, is included in Figure 2.4.

Figure 2.4: PhD research questions and relevant data sources



Chapter 3: Systematic review to investigate changes in non-vaccine HPV types following HPV vaccination

3.1. Introduction

In this chapter, I present a systematic review and meta-analysis which I conducted to investigate the population-level impact of national HPV vaccination on non-vaccine HPV types (Figure 2.4; research question 2). Originally, at the start of this PhD, I had intended to conduct a review to compare the prevalence of high-risk HPV infection (including vaccine HPV types and cross-protective types) between the pre- and post-vaccination time periods. After developing the research question, search strategy and inclusion criteria for this systematic review, I learned that another research group (Université Laval, Canada) were conducting a similar systematic review of the population level impact of HPV vaccination on infection and early disease outcomes. I agreed to provide data from England to inform their review and I was invited to be an author. This group were investigating changes in the combined prevalence of any high-risk HPV type, the combined prevalence of vaccine HPV types and the combined prevalence of cross-protective HPV types. The group were not considering changes in individual HPV types. I therefore amended my research question slightly to examine the evidence for changes in HPV infection with individual non-vaccine high-risk HPV types. I also adapted my inclusion criteria slightly to agree with those being used by Université Laval so that these systematic reviews would complement each other (Table A1 in the Appendix). I assessed the eligibility of studies that were identified using my original search criteria; as a result one additional study was included in the systematic review being led by Université Laval.

The manuscript below published in Emerging Infectious Diseases in 2016 provides the results of this systematic review and meta-analysis which I conducted and

analysed to investigate changes in non-vaccine HPV types after HPV vaccine introduction. The supplementary material for this publication is included in Appendix B. As the published review was based on a search conducted in February 2016, I re-ran the systematic review in December 2017 for the purposes of this thesis. I summarise the findings of this update at the end of this Chapter.

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RESEARCH PAPER COVER SHEET

PLEASE NOTE THAT A COVER SHEET MUST BE COMPLETED FOR EACH RESEARCH PAPER INCLUDED IN A THESIS.

SECTION A – Student Details

Student	David Mesher
Principal Supervisor	Sara Thomas
Thesis Title	Assessment of the population-level impact of a high coverage HPV immunisation programme in young females

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

Where was the work published?	Emerging Infectious Diseases		
When was the work published?	October 2016		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	N/A		
Have you retained the copyright for the work?*	**Yes**	Was the work subject to academic peer review?	**Yes**

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SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	
Please list the paper's authors in the intended authorship order:	
Stage of publication	Choose an item.

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	Originally, I developed the proposal (with input from Kate Soldan and Sara Thomas) to perform a systematic review to consider changes in HPV DNA prevalence following national introduction of HPV vaccination. In the process of designing the protocol for this systematic review, I was approached by another research group requesting our data

	for a similar review considering changes in vaccine-types and HPV types thought to have some cross protection. In response to this, I changed the focus of our systematic review and meta-analysis to focus on changes in individual non-vaccine HPV types. I developed the protocol for the data collection and analysis. Where possible, this was aligned with the review conducted by the other research group. I designed the search strategy with guidance from Sara Thomas. I conducted the search and de-duplicated between different search databases. I searched abstracts to identify potentially eligible papers and then performed the full paper search. Eligible papers were compared with those identified by the other research group and any additional papers which I identified were shared with them prior to publication. Once all eligible papers were identified, I extracted available information from the manuscripts. Type-specific data were not available in any of the eligible papers hence I contacted ten corresponding authors to request these data. I conducted all data management and analysis and produced the first draft of the manuscript which was commented on by all authors. This paper was peer reviewed and I incorporated suggestions from reviewers and responded to their comments, with input from other authors.

Student Signature: [redacted] **Date:** 12/04/2018

Supervisor Signature: [redacted] **Date:** 13/04/18

SYNOPSIS

Population-Level Effects of Human Papillomavirus Vaccination Programs on Infections with Nonvaccine Genotypes

David Mesher, Kate Soldan, Matti Lehtinen, Simon Beddows, Marc Brisson, Julia M.L. Brotherton, Eric P.F. Chow, Teresa Cummings, Mélanie Drolet, Christopher K. Fairley, Suzanne M. Garland, Jessica A. Kahn, Kimberley Kavanagh, Lauri Markowitz, Kevin G. Pollock, Anna Söderlund-Strand, Pam Sonnenberg, Sepehr N. Tabrizi, Clare Tanton, Elizabeth Unger, Sara L. Thomas

We analyzed human papillomavirus (HPV) prevalences during prevaccination and postvaccination periods to consider possible changes in nonvaccine HPV genotypes after introduction of vaccines that confer protection against 2 high-risk types, HPV16 and HPV18. Our meta-analysis included 9 studies with data for 13,886 girls and women ≤19 years of age and 23,340 women 20–24 years of age. We found evidence of cross-protection for HPV31 among the younger age group after vaccine introduction but little evidence for reductions of HPV33 and HPV45. For the group this same age group, we also found slight increases in 2 nonvaccine high-risk HPV types (HPV39 and HPV52) and in 2 possible high-risk types (HPV53 and HPV73). However, results between age groups and vaccines used were inconsistent, and the increases had possible alternative explanations; consequently, these data provided no clear evidence for type replacement. Continued monitoring of these HPV genotypes is important.

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Persistent infection with a high-risk human papillomavirus (HPV) genotype is necessary for development of cervical cancer (*1*). Two high-risk types, HPV16 and HPV18, cause ≈70%–80% of cervical cancers (*2–4*). The HPV vaccines currently available commercially have been shown in trial settings to have ≈100% vaccine efficacy against cervical disease caused by vaccine-specific high-risk HPV types: bivalent and quadrivalent vaccines against HPV16 and HPV18 and the new nonavalent vaccine against HPV31, HPV33, HPV45, HPV52, and HPV58 (*5–7*). Clinical trial data for the bivalent and quadrivalent vaccines have shown low-to-moderate protection (i.e., cross-protection) against other high-risk HPV types that are phylogenetically related to HPV16 and HPV18 (*8,9*).

Many countries have now introduced HPV vaccination programs (*10*). A recently published systematic review and meta-analysis assessed population-level effects of HPV vaccination on vaccine HPV types and showed strong evidence that HPV vaccination is highly effective against infections with these vaccine-specific high-risk types (*11*). The review also examined closely related HPV types as a single group and found evidence of cross-protection overall in a population-based setting (*11*). However, assessment of changes in the prevalence of closely related HPV types combined may not provide full evidence of the effects of a national vaccination program because examining the types as a single group potentially conceals decreases or increases in the prevalence of individual types. Grouping HPV types together limits the possibility of examining

cross-protection provided by specific HPV types and of detecting changes in other individual nonvaccine types. For example, a theoretical concern is that reduced prevalences of infection with HPV16 and HPV18 could lead to other high-risk HPV types occupying those niches and becoming more common causes of disease. Although type replacement was not observed in the clinical trials (*12*), monitoring for possible type replacement in population-based settings after the introduction of national HPV vaccination programs is important. Furthermore, because nonvaccine HPV types are far less common than vaccine HPV types, a single study may have limited scope to determine whether type replacement has occurred. Combining data from several reports improves the ability to investigate type replacement. We aimed to investigate population-level effects of HPV vaccination programs that used bivalent or quadrivalent vaccines on type-specific prevalences of infection caused by individual nonvaccine high-risk HPV types.

Methods

Objectives

Using data from surveys conducted before an HPV vaccination program was introduced and data from surveys after the program was introduced, we compared HPV prevalences for similar populations within the same country. We conducted a systematic literature search to determine changes in HPV prevalence for each nonvaccine high-risk HPV type. At the time of our search, any eligible study would have considered vaccination that used bivalent or quadrivalent vaccines; consequently, high-risk HPV types used only in the nonavalent vaccine were considered nonvaccine HPV types. Each individual type was presented separately in our analysis. We included HPV types for which some cross-protection had been demonstrated in clinical trials (HPV31 and HPV33, which are phylogenetically related to HPV16, and HPV45, which is phylogenetically related to HPV18) (*8,9,13*); other high-risk HPV types included in the nonavalent vaccine (HPV52 and HPV58); other high-risk and probably high-risk HPV types (HPV35, HPV39, HPV51, HPV56, HPV59, and HPV68); and other possibly high-risk HPV types (HPV26, HPV53, HPV70, HPV73, and HPV82), as classified by the International Agency for Research on Cancer (*14*). This systematic review and meta-analysis was reported in accordance with PRISMA guidelines (*15*).

Search Strategy and Selection Criteria

Using Embase, Medline, LILACS, and African Index Medicus databases, we searched for eligible publications published from 2007, the year that the first HPV vaccination programs were introduced, through February 19, 2016. To identify relevant studies that mentioned both vaccination and HPV infection or a related disease (such as HPV-related precancerous lesions, cancers, and genital warts), the search strategy incorporated MeSH terms from the PubMed database (http://www.ncbi.nlm.nih.gov/mesh) and relevant words found in the title or abstract (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/10/16-0675-Techapp1.pdf). The search had no language restrictions.

Eligible studies were those that assessed population-level effects of HPV vaccination over time by comparing the prevalence of HPV infection (defined by the detection of HPV DNA in patient samples) during a prevaccination period with the prevalence during a postvaccination period. We excluded studies comparing HPV infection in vaccinated persons with HPV infection in unvaccinated persons as part of an individually randomized trial because such studies would not measure population-level effects. Similarly, we excluded studies in which HPV infection was compared only between unvaccinated and vaccinated persons in the postvaccination period. We also excluded studies in which only a small proportion (<2%) of the postvaccination study population was vaccinated (i.e., studies conducted in largely unvaccinated populations). One author (D.M.) initially reviewed titles and abstracts of studies for eligibility; we reviewed in full those studies that appeared to address changes in HPV prevalence after introduction of HPV vaccination programs. We also compared search results with those identified in a recent related review (*11*), which compared prevaccination and postvaccination periods for high-risk vaccine types (HPV16 and HPV18), cross-protected types (HPV31, HPV33, and HPV45), and all high-risk HPV nonvaccine types combined.

Data Extraction and Data Quality

For each study, we extracted data on study design and country of study. Then, for both prevaccincation and postvaccination periods, we extracted data on year(s) of sample collection, study setting and population, sample size, specimen type, assay used for HPV DNA testing, HPV genotypes included in the assay, demographic and sexual behavior data collected, and the measure of effect (and method used to determine any effect). For the postvaccination period, we also extracted data on the method used to ascertain estimated vaccination coverage.

In addition, we assessed the potential bias in each study by considering the comparability of the study populations in the prevaccination versus postvaccination periods (i.e., similar setting and population demographics); the extent of adjustment for potential confounders; the suitability of the specimen type to assess HPV DNA infection; the suitability of the assay used for accurate HPV DNA testing (and whether the suitability of assays differed between the prevaccination and postvaccination periods); and the method used to estimate HPV vaccination coverage. To assess

external validity, we considered whether the study samples were population based. Each of these factors was scored as either low risk or high risk.

When published data on HPV prevalence and prevalence ratios (PRs) for individual high-risk HPV types were unavailable, we contacted authors to request the HPV type-specific prevalences during the prevaccination and postvaccination periods and the PRs for the 2 periods for each nonvaccine high-risk HPV type. We requested PRs adjusted for demographic and sexual behavior data or the unadjusted PRs if data on confounders were unavailable; we calculated unadjusted PRs if authors provided raw data. By using data from a previously conducted validation study, 1 study included adjusted odds ratios rather than PRs to adjust for the change in assay used during the prevaccination and postvaccination periods (*16*).

Data Analysis

We used estimates weighted to account for selection processes if that data were available from authors unweighted numbers, as shown in online Technical Appendix Table 1). We also stratified data by age group (i.e., ≤19 and 20–24 years of age) because of expected lower rates of vaccination coverage and lower vaccine effectiveness in those vaccinated at older ages. Consequently, for each study, we requested data from authors for the same 2 age groups. One study included data for girls <13 years of age, so we requested data restricted to those 16–19 years of age (*17*).

To enable calculation of a PR for a prevalence of 0 during either the prevaccination or postvaccination period, we used a continuity correction of 0.5. When prevalence was 0 for both the prevaccination and postvaccination periods, we excluded the study from the meta-analysis for the relevant age group and HPV type. Results were further stratified by type of vaccine used (i.e., bivalent or quadrivalent). PRs within each subgroup were combined to obtain a summary PR by using a fixed-effects model if data were not shown to be heterogeneous; lack of heterogeneity was determined by a p value ≥0.10 calculated with the Cochrane Q test or by an I^2 value <25% (*18*). Sensitivity analyses were restricted to studies that used cervical, vulval, or vaginal swabs as specimen type because urine samples have lower sensitivity for detecting HPV DNA infection (*19*).

Results

Included Studies

After we eliminated duplications, we identified 4,648 unique articles in searches from all 4 databases (Figure 1). An initial search of title and abstracts of these articles excluded 4,508 (97.0%) because of ineligibility. For the remaining 140 articles, we examined the full text to determine compliance with eligibility criteria and identified 10 eligible studies (Figure 1). Of these 10 studies, 1 met all eligibility criteria, but the type-specific PRs were unavailable from authors (*20*). Therefore, we included 9 studies in the systematic review and meta-analysis (*16,17,21–27*). All eligible studies were repeat cross-sectional studies that compared changes in prevalence in populations before and after introduction of a national HPV vaccination program (online Technical Appendix Table 1). Because only 1 study considered changes in HPV infection among male and female populations, we considered only female populations in the analysis. Two studies were population-based national surveys (*23,26*); 3 studies were conducted among young women obtaining chlamydia screening (*16,17,27*); 2 studies comprised young women attending a primary care clinic, community health center, or hospital-based adolescent clinic (*21,22*); and 2 studies comprised women obtaining cervical screening (*24,25*) (online Technical Appendix Table 1). The included studies contained data on 13,886 girls and women ≤19 years of age and 23,340 women 20–24 years of age.

The studies varied in methodologic quality on the basis of potential bias (Table 1). Most studies collected some demographic and sexual behavior data to enable appropriate

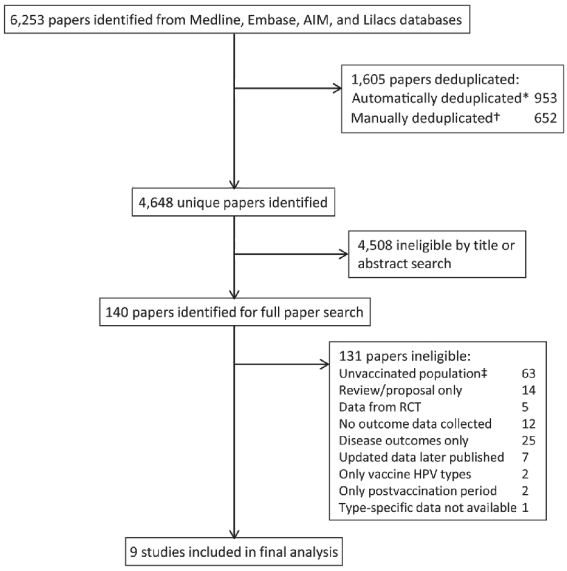

Figure 1. Flowchart for eligible studies included in systematic review and meta-analysis of changes in prevalences of nonvaccine human papillomavirus (HPV) genotypes after introduction of HPV vaccination. *100% title match, author's surname and initial, publication year, and periodical; †85% title match, and author surname; ‡includes studies in which the vast majority of the population were unvaccinated. RCT, randomized controlled trials.

Table 1. Potential bias and external validity of studies included in meta-analysis of changes in prevalences of nonvaccine HPV genotypes*

Potential bias factors	Study, authors (reference no.)								
	Mesher et al. (*16*)	Söderlund-Strand et al. (*17*)	Cummings et al. (*21*)	Kahn et al. (*22*)	Sonnenberg et al. (*23*)	Tabrizi et al. (*24*)	Cameron et al. (*25*)	Markowitz et al. (*26*)	Chow et al. (*27*)
Population-based samples†	**H**	**H**	**H**	**H**	L	L	L	L	**H**
Comparative populations†	**H**	**H**	L	L	L	L	L	L	**H**
Risk factor data collected and adjusted for	**H**	**H**	L	L	L	**H**	**H**	L	L
Samples suitable for assessing HPV	L	L	L	L	**H**	L	L	L	L
Assay with suitable accuracy	L	L	L	L	L	L	L	L	L
Identical HPV assays†	**H**	L	L	L	L	L	L	L	L
Vaccination status collected	**H**	**H**	L	L	**H**	L	L	**H**	**H**

*HPV, human papillomavirus; H (in bold), high risk of bias; L, low risk of bias.
†For both prevaccination and postvaccination periods.

adjustment of the relative risks, although the number of factors collected was limited in some studies (*16,17,24,25*) (Table 1; online Technical Appendix Table 1).

HPV Types Included in Nonavalent HPV Vaccines

HPV Types with Prior Evidence for Cross-Protection

We found evidence of reduced prevalence of HPV31 (Figure 2; Table 2) among girls and women ≤19 years of age (PR 0.73, 95% CI 0.58–0.91) but found little evidence of changed prevalences for HPV33 or HPV45 among this age group (PR 1.04, 95% CI 0.78–1.38 for HPV33; PR 0.96, 95% CI 0.75–1.23 for HPV45). Results were heterogeneous for HPV31, HPV33, and HPV45 in women 20–24 years of age; consequently, we did not calculate summary PRs (Figure 2; Table 2).

Other HPV Types

We found evidence of increased prevalence of HPV52 in those ≤19 years of age (PR 1.34, 95% CI 1.13–1.59) (Figure 3; Table 2), but because of heterogeneity, we did not calculate summary PRs for those 20–24 years of age. We found no evidence of a changed prevalence for HPV58 among the younger age group (PR 1.01, 95% CI 0.80–1.26) but found borderline evidence of an increase for those 20–24 years of age (PR 1.14, 95% CI 0.99–1.31).

Other High-Risk and Possibly High-Risk HPV Types

No consistent patterns appeared across the studies for other HPV vaccine types not used in the nonavalent vaccine (Table 2; online Technical Appendix Figure 1). We found evidence of increased prevalences from the prevaccination period to the postvaccination period in those ≤19 years of age for HPV39 (PR 1.27, 95% CI 1.05–1.54), HPV53 (PR 1.51, 95% CI 1.10–2.06), and HPV73 (PR 1.36, 95% CI 1.03–1.80). For women 20–24 years of age, evidence indicated increased prevalence for HPV39 (PR 1.13, 95% CI 1.00–1.28).

Sensitivity Analysis

As a sensitivity analysis, we performed 3 additional analyses, all stratified by age group: by type of vaccine used (i.e., bivalent or quadrivalent); by potential bias of the original study (i.e., relatively low potential bias, defined as <3 factors indicating high risk of bias; or relatively high potential bias, defined ≥3 factors indicating high risk of bias) (Table 1); and by vaccination coverage (i.e., low <50%; high ≥50%). For studies in settings that used the bivalent vaccine, we found evidence of increased prevalence between the prevaccination period and postvaccination periods among those ≤19 years of age for HPV52, HPV53, HPV56, and HPV70 (online Technical Appendix Table 2, Figures 2–4). Prevalence of HPV53 among women 20–24 years of age also increased. For the quadrivalent vaccine, evidence showed increased prevalences of HPV39, HPV51, and HPV59 for those ≤19 years of age. Among those 20–24 years of age, evidence indicated increased prevalence of HPV52 and HPV70 (online Technical Appendix Table 2, Figures 2–4).

Many of our analyses that were stratified by potential bias of the included studies had results similar to those in the unstratified analyses (online Technical Appendix Table 3). However, among those ≤19 years of age, studies with a relatively low potential bias showed no evidence of increased prevalence for HPV52 or HPV39, although evidence existed when the studies were unstratified. For studies with relatively high potential bias, among this younger age group, evidence showed increased prevalences of HPV51 and HPV70, although these increases were not present in the unstratified analysis. In women 20–24 years of age, evidence showed decreased prevalence for HPV33 in those studies with a relatively low potential bias. No

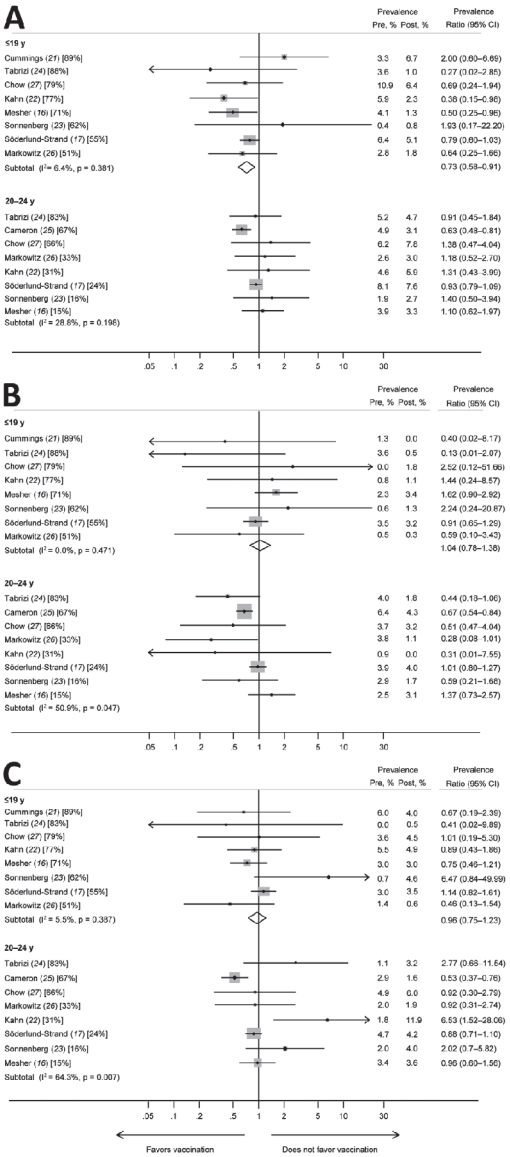

Figure 2. Prevalence ratios and 95% CIs for high-risk human papillomavirus (HPV) types (HPV31, HPV33, and HPV45) that had evidence of cross-protection for girls and women ≤19 years of age and women 20–24 years of age in studies included in a meta-analysis of changes in prevalences of nonvaccine HPV genotypes after introduction of HPV vaccination. A) HPV31; B) HPV33; C) HPV45. Percentages in brackets represent vaccination coverage (≥1 dose) for each study and age group. The size of the gray boxes around the plot points indicates the relative weight given to each study in the calculation of the summary estimate. The study by Cameron et al. (*25*) is omitted from analyses for the younger age group because this study included no data for the group ≤19 years of age. The study by Cummings et al. (*21*) is omitted from analyses for women 20–24 years of age because this study included no data for this age group. Pre, prevaccination; post, postvaccination.

summary estimate was provided in the unstratified analysis because of heterogeneity of data. Studies with a relatively high potential bias showed evidence of increased prevalences of HPV52 and HPV58 among women 20–24 years of age. Among this older age group, evidence existed for decreased prevalence of HPV82 in those studies with both relatively high potential bias and relatively low potential bias, although those studies with relatively high potential bias had a larger decrease. Again, no summary estimate was provided in the unstratified analysis because of heterogeneity.

Vaccination coverage was high for the younger age group in all studies (online Technical Appendix Table 4). For the older age group, studies with high vaccination coverage showed decreased prevalence for HPV31. No summary estimate was provided for the unstratified analysis because of heterogeneity. For the older age group, we found evidence of increased prevalences for HPV39 and HPV58 (similar to results from the unstratified analysis) but only in studies with low vaccination coverage. Although not seen in the unstratified analysis, we also found evidence of an increased prevalence for HPV70 in low-coverage studies and borderline evidence of an increased prevalence for HPV26 in high-coverage studies. No summary estimates were provided for the unstratified analyses because of heterogeneity.

Discussion

Comprehensive postvaccination surveillance should not only consider reductions of vaccine type–specific infection and associated disease but should also assess any other potential effects of reductions of targeted infections. We assessed changes in nonvaccine HPV types to determine evidence of cross-protection for individual HPV types and to investigate the potential concern that reductions in certain HPV types after the introduction of HPV vaccination in a population could create a niche that enables other nonvaccine high-risk HPV types to become more common (i.e., type replacement). We found evidence of a reduction in the prevalence of HPV31 among girls and women ≤19 years of age. Our main analysis showed increases in other nonvaccine HPV types (HPV39, HPV52, HPV53, HPV58, and HPV73), but these increases were inconsistent for the 2 age groups examined and the vaccines used.

A previous systematic review evaluated changes in high-risk HPV types combined and found evidence of a reduction in the prevalence of HPV types closely related to vaccine types (HPV31, HPV33, and HPV45) when they were considered as a single group (PR 0.72, 95% CI 0.54–0.96 for girls and women 13–19 years of age) (*11*). Our review provides evidence of reduced prevalence for HPV31 but little evidence of reduced prevalence for HPV33 or HPV45.

Table 2. Summary prevalence ratios for meta-analysis of changes in nonvaccine high-risk HPV types among girls and women, by age group*

Population age group, y, and HPVtype	No. studies†	Heterogeneity		Prevalence ratio (95% CI)
		I^2, %	p value	
≤19				
HPV types in nonavalent vaccine	8			
HPV31		6.4	0.381	0.73 (0.58–0.91)
HPV33		0	0.471	1.04 (0.78–1.38)
HPV45		5.5	0.387	0.96 (0.75–1.23)
HPV52		24.0	0.238	1.34 (1.13–1.59)
HPV58		0	0.727	1.01 (0.80–1.26)
Other high-risk HPV types	8			
HPV35		25.1	0.229	–
HPV39		0	0.984	1.27 (1.05–1.54)
HPV51		43.6	0.088	–
HPV56		74.3	<0.001	–
HPV59		66.8	0.004	–
HPV68		0	0.690	1.26 (0.88–1.81)
Other possibly high-risk HPV types	6			
HPV26		0	0.478	1.63 (0.84–3.16)
HPV53		3.6	0.394	1.51 (1.10–2.06)
HPV70		23.6	0.257	1.34 (0.75–2.39)
HPV73		0	0.961	1.36 (1.03–1.80)
HPV82		49.0	0.081	–
20–24				
HPV types in nonavalent vaccine	8			
HPV31		28.8	0.198	–
HPV33		50.9	0.047	–
HPV45		64.3	0.007	–
HPV52		31.0	0.180	–
HPV58		0	0.806	1.14 (0.99–1.31)
Other high-risk HPV types	8			
HPV35		7.9	0.369	1.07 (0.85–1.34)
HPV39		0	0.522	1.13 (1.00–1.28)
HPV51		49.8	0.052	–
HPV56		82.6	<0.001	–
HPV59		63.6	0.007	–
HPV68		35.6	0.145	–
Other possibly high-risk HPV types	6			
HPV26		44.3	0.110	–
HPV53		30.8	0.204	–
HPV70		25.1	0.246	–
HPV73		59.2	0.032	–
HPV82		38.3	0.151	–

*HPV, human papillomavirus; –, prevalence ratio not calculated because of heterogeneity of data.
†Number of studies was the same for all HPV types within each category.

Comparing HPV prevalence in a prevaccination period to prevalence in a similar population in a postvaccination period enables consideration of population-level effects of HPV vaccination on HPV prevalence. However, these repeat cross-sectional study designs have limitations. Although all studies addressed similar populations in the prevaccination and postvaccination periods, these populations may have undergone temporal changes that are independent of HPV vaccination over time and that possibly affect HPV prevalence. For example, increases in diagnoses of other sexually transmitted infections have occurred during the same period as that of HPV vaccination programs (*28*). Furthermore, incidence of genital warts increased in many countries before vaccine introduction (*29–31*) and has continued to increase postvaccination in persons ineligible for vaccination (*11*). Such findings suggest that the increases we observed in some HPV types are possibly associated with broad increases in sexual risk over time. We considered changes in demographics and sexual behavior for the populations over time when information was available, but unrecorded population changes or other temporal changes affecting the relative proportions of high-risk HPV types likely occurred over time (*32,33*). Also, more geographic variation exists in the relative frequency of nonvaccine HPV types in populations compared with the prevalence of HPV16, which, before the vaccination programs, was the most frequent high-risk type observed in almost all populations (*34*).

Furthermore, the change in assay used during the prevaccination and postvaccination periods was a potential source of bias in 1 study (*16*), which calculated odds ratios (ORs) adjusted for differences in diagnostic accuracy. This adjusted OR could not be converted to a PR by using the log-binomial model and was included as an OR. However,

given the low prevalence of individual HPV types, the use of an OR instead of a PR for this study was unlikely to have affected the results substantially.

Another limitation is that the broad-spectrum assays used in these studies (and in baseline prevaccination evaluations globally) can lack sensitivity for detecting individual HPV types when multiple types are present, particularly if another HPV type with a higher viral load is present. In the postvaccination period, in the absence of HPV16 and HPV18, this lack of sensitivity could lead to an apparent but artificial increase in nonvaccine types because these types were underestimated in the prevaccine period because of the predominance of HPV16 or HPV18. Studies have shown this potential unmasking effect (*35,36*); some increases in nonvaccine types that we observed could result from unmasking.

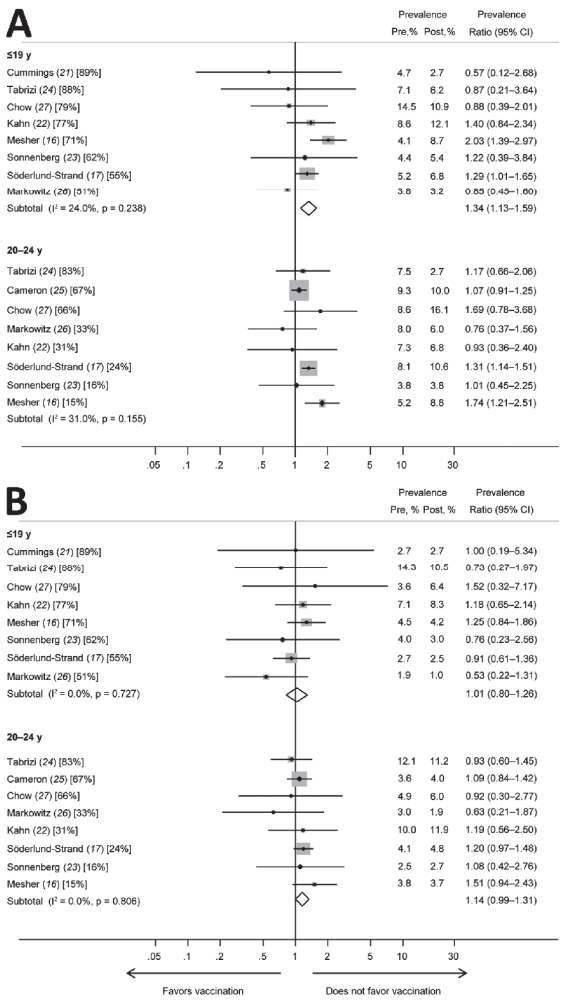

Figure 3. Prevalence ratios and 95% CIs for other high-risk human papillomavirus (HPV) types (HPV52 and HPV58) included in the nonavalent vaccine for girls and women ≤19 years of age and women 20–24 years of age in studies included in a meta-analysis of changes in prevalences of nonvaccine HPV genotypes after introduction of HPV vaccination. A) HPV52; B) HPV58. Percentages in brackets represent vaccination coverage (≥1 dose) for each study and age group. The sizes of the gray boxes around the plot points indicates the relative weight given to each study in the calculation of the summary estimate. The study by Cameron et al. (*25*) is omitted from analyses for the younger age group because this study included no data for persons ≤19 years of age. The study by Cummings et al. (*21*) is omitted from analyses for women 20–24 years of age because the study included no data for this age group. Pre, prevaccination; post, postvaccination.

Given the low prevalence of some nonvaccine HPV types, assessing changes in prevalence for individual types since the introduction of HPV vaccination has been challenging. By combining data from several studies, we enhanced our power to consider changes in individual HPV types. However, even with data from 13,886 girls and women ≤19 years of age and 23,340 women 20–24 years of age, we still had limited power to consider changes in very rare HPV types or to investigate reasons for the heterogeneity in findings for some HPV types because of inconsistent evidence for increases of specific nonvaccine types between age groups and the 2 (i.e., bivalent and quadrivalent) vaccines. Conversely, type 1 errors can occur with multiple testing, so modest evidence for increases should be interpreted with caution.

We decided against performing random-effects meta-analyses in the presence of between-study heterogeneity because, in most instances, inconsistency occurred in the direction of effect, making a summary estimate (i.e., the average value of these opposing effects) uninformative (*37*). Exploring the causes of heterogeneity could provide further insight into the reasons for these increases, so we performed 3 subgroup analyses by vaccine used, potential bias, and vaccine coverage. Results of the stratification by potential bias suggested that increased PRs for some HPV types may have been reported more often in the studies with relatively high potential bias. However, for all 3 subgroup sensitivity analyses, the small number of studies in each stratum limited the interpretation of the analyses. Similarly, we were limited to only 8 studies for each age group and had insufficient ability to perform meta-regression analyses (because meta-regression should generally not be considered for <10 studies) (*37*). As further data accrue, a useful future analysis would be exploring the association between reductions in the HPV vaccine types and any increases (not resulting from unmasking) in nonvaccine HPV types. If increases result from type replacement, then we would expect to see increasing prevalences of nonvaccine HPV types as prevalences of vaccine HPV types decrease.

Our confirmation of reductions in a cross-protected HPV type is encouraging. However, the results of this systematic review and meta-analysis provide no clear evidence

for type replacement because the data are unclear about the extent to which any observed increases result from other temporal changes, changes in the study populations, or an unmasking effect of broad spectrum HPV assays. Large-scale epidemiologic analyses that use various designs have not detected evidence of any interactions between high-risk types, and the known high evolutionary stability of these viruses lessens the risk that type replacement will be a problem (*38*,*39*).

Most women included in the surveillance studies were those vaccinated at older ages (i.e., potentially vaccinated after HPV exposure), and some studies included populations with relatively low coverage, compared with nationally reported vaccination coverage for routine cohorts. Future studies should continue to monitor population-level prevalences of these HPV types. In particular, studies should consider populations vaccinated at young ages and having high vaccination coverage and, perhaps more important, should examine the absolute prevalence of cervical intraepithelial neoplasia 3 lesions attributed to each high-risk HPV type.

Acknowledgments

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Mr. Mesher is a senior scientist at Public Health England. His work involves monitoring and evaluating the population-level effects of the national HPV vaccination program.

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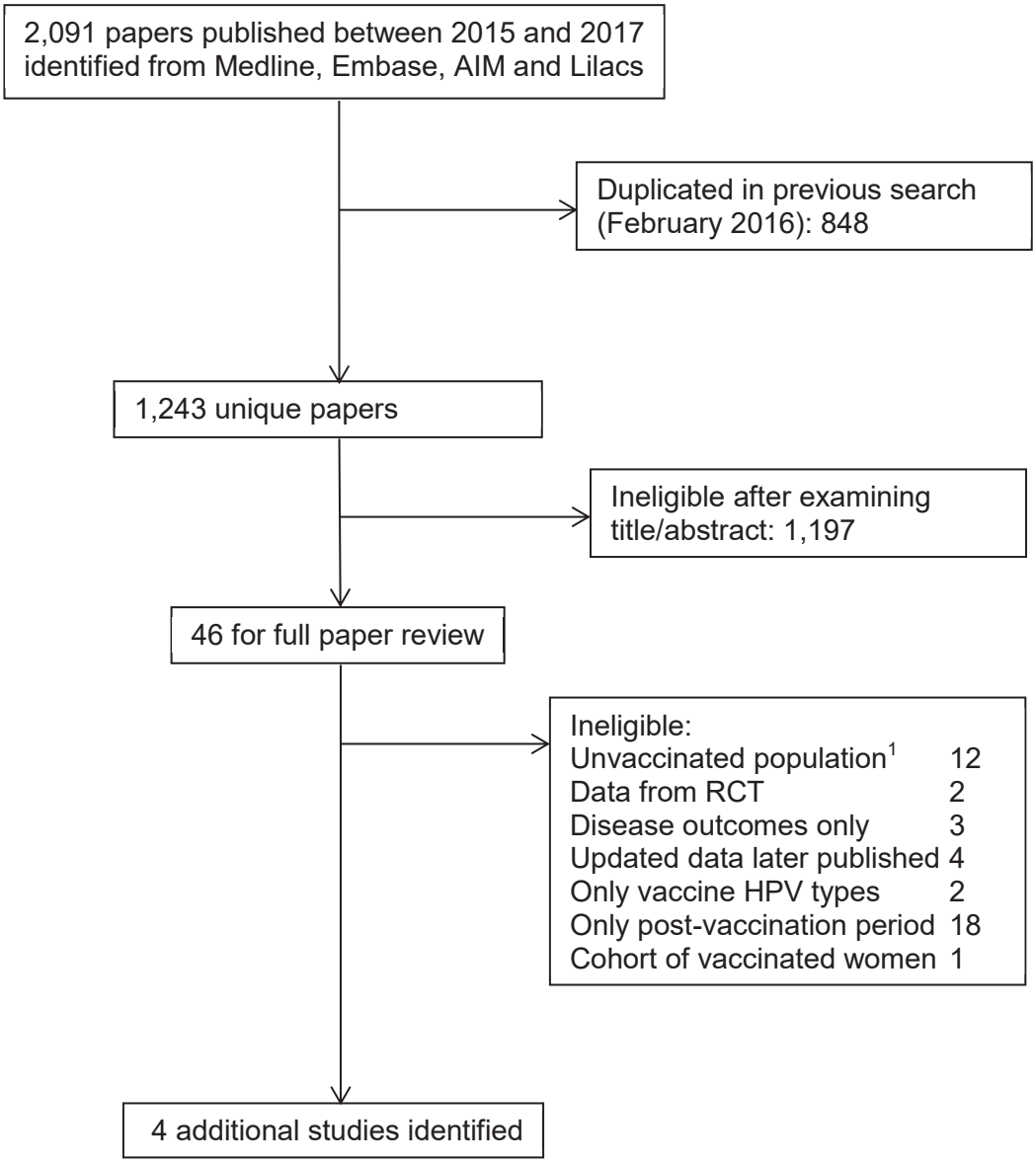
3.3. Update to previous analysis

The database search for this systematic review and meta-analysis presented in the previous section was conducted in February 2016. I re-ran the database searches on 4th December 2017 using the same search criteria with the exception that I restricted to publications in 2015, 2016 and 2017. I included 2015 to identify any publications which were published in 2015 but had not been updated on Medline or Embase in February 2016 when the previous search was conducted. To ensure that publications were not included multiple times, I first deduplicated between Embase and Medline, as before, and then deduplicated with the previous search conducted in February 2016. A total of 1,243 new and unique publications were identified for eligibility screening (Figure 3.1).

Eligibility criteria were identical to those used for the published systematic review. I searched all titles/abstracts and identified 46 potentially eligible publications for a full paper search. Of these, four publications were eligible for inclusion (three of which were updated versions of publications included in the previous review conducted in February 2016). I provide the reasons for exclusion in Figure 3.1 and details of the included papers in Table 3.1.

Figure 3.1: Flow chart for eligible studies included in the update of the systematic review (conducted 4th December 2017)



1: Includes studies where the vast majority of the population were unvaccinated

Table 3.1: Characteristics of studies selected for systematic review

Study	Country (vaccine type)	Years of specimen collection	Number of specimens tested	Study population and setting	Specimen type	Assay for HPV DNA testing	Demographic and sexual behaviour data collected	Vaccination status
Studies included in original systematic review (conducted February 2016)								
Cameron et al[85]	See Technical Appendix Table 1 in Appendix B							
Chow et al[86]								
Cummings et al[87]								
Kahn et al[88]								
Markowitz et al[89]								
Mesher et al[90]								
Söderlund-Strand et al[91]								
Sonnenberg et al[26]								
Tabrizi et al[92]								
Studies identified in initial systematic review (conducted February 2016) but excluded as type-specific data were not available								
Dunne et al[93]	USA (quadrivalent)	Pre-vacc: 2007 Post-vacc: 2012-2013	Pre-vacc: 4,138 Post-vacc: 4,171	Women aged 20-29 years old attending for routine cervical cancer screening at a clinic in Northwest USA	Residual LBC specimen	Linear Array HPV Genotyping test	Age at first dose, race, ethnicity, family poverty, tested for chlamydia in past year, tested positive for chlamydia in past year, tested for HIV in past year, tested for pregnancy in past 6 months	Extracted from electronic medical record

Study	Country (vaccine type)	Years of specimen collection	Number of specimens tested	Study population and setting	Specimen type	Assay for HPV DNA testing	Demographic and sexual behaviour data collected	Vaccination status
Updated data from studies included in initial systematic review								
Kahn et al[94] (update of Kahn et al[88])	USA (quadrivalent)	Pre-vacc: 2006-2007 Post-vacc: 2009-2010 2013-2014	Pre-vacc: 371 Post-vacc: 409 400	Females (aged 13-26 years old) who had history of sexual intercourse, recruited from hospital based adolescent clinic and a community health centre	Cervicovaginal swabs by clinician or self-collected swab	Linear Array HPV Genotyping test	Age, race, health care insurance, knowledge about HPV vaccines, smoking status, gynaecologic history (number of pregnancies, history of STIs), sexual behaviours (age at first sex, number of male lifetime partners, number of male partners in previous 3 months, anal sex, condom use) Comparisons were adjusted for propensity score to account for confounding	Self-administered questionnaire
Kavanagh et al[95] (update of Cameron et al[85])	UK: Scotland (Bivalent)	Pre-vacc: 2009-2010 Post-vacc: 2011-2015	Pre-vacc: 2,757 Post-vacc: 5,827	Females (aged 20-21 years old) attending for cervical screening as part of national cervical screening programme.	Residual LBC specimen	Multimetrix HPV assay	Scottish Index of Multiple Deprivation, month/year of birth, age at vaccination	Data linked from Scottish Immunisation Recall System

Study	Country (vaccine type)	Years of specimen collection	Number of specimens tested	Study population and setting	Specimen type	Assay for HPV DNA testing	Demographic and sexual behaviour data collected	Vaccination status
Updated data from studies included in initial systematic review (continued)								
Oliver et al[96] (update of Markowitz et al[89])	USA (Quadrivalent)	Pre-vacc: 2003-2006 Post-vacc: 2009-2012	Pre-vacc: 1,795 Post-vacc: 2,424	Females (aged 14-24 years old) participating in population based NHANES survey	Self-collected cervicovaginal sample	Linear Array HPV Genotyping test	Ethnicity, poverty index and, for those reported ever having sex; age at first sex, lifetime number of partners, number of partners in the previous 12 months. Prevalence ratios were adjusted for race/ethnicity, poverty index, and number of lifetime partners	Self-reported
New studies identified from update of systematic review								
Grun et al[97]	Sweden (majority quadrivalent)	Pre-vacc: 2008-2011 Post-vacc: 2013-2015	Pre-vacc: 615 Post-vacc: 338	Females (aged 15-23 years old) attending a youth health clinic in Sweden	Cervical samples by professional or self-test	Multiplex polymerase chain reaction and Luminex technology	Age. No other data reported	Self-reported

To summarise, three of the four additional papers reported updates to results that were included in the original review. Kahn et al compared HPV prevalence at three separate time points (2006-2007 (prior to HPV vaccination); 2009-2010 and 2013-2014) among 13-26 year old women in the USA[94]. This was an update of a previous paper by Kahn et al published in 2012 which compared HPV prevalence for the first two time periods only and showed a reduction in the prevalence of HPV vaccine types but a slight increase in non-vaccine HPV types[88]. In the updated publication, the authors demonstrated a continued decline in the vaccine-types but, contrary to the previous publication, the prevalence of non-vaccine HPV types in 2013-2014 was similar to the prevalence in 2006-2007. This updated publication did not include investigation of changes in any individual HPV types. Kavanagh et al[95] published an update of a previous analysis published by Cameron et al[85], which was conducted among 20-21 year old women attending for cervical screening in Scotland between 2009 and 2013. The previous analysis demonstrated a decrease in HPV16, 18, 31, 33 and 45 but a non-significant increase in HPV51. In the updated publication, extended to 2015, these results were strengthened as the authors demonstrated declines in prevalence of vaccine types from 28.9% in 2009 to 4.8% in 2015 and substantial declines in HPV31, 33 and 45 over the same time period (13.0% to 3.0%). Despite these clear declines in later birth cohorts for HPV16, 18, 31, 33 and 45, there was no evidence of any trends in the prevalence of other high-risk HPV types which would suggest type-replacement (the authors presented graphically the prevalence of individual HPV types by birth cohort in the online appendix). Oliver et al[96], updated a previous paper published by Markowitz[89], considering HPV prevalence among women included in the National Health and Nutrition Examination Survey (NHANES), an ongoing series of cross-sectional surveys which are designed to be nationally representative of the US population (this was a separate population from the one considered by Kahn et al above). The previous analysis, which compared pre-vaccination HPV prevalence

(specimens collected between 2003 and 2006) to post-vaccination HPV prevalence (specimens collected between 2009 and 2012), demonstrated reductions in the HPV vaccine types but no significant changes in other HPV types. The updated analysis extended post-vaccination data collection to 2014 and demonstrated a decline in vaccine types from 11.5% in 2003-2006 to 3.3% in 2011-2014. This publication included individual changes in the prevalence of 12 high-risk HPV types and a further 21 other HPV types. The authors concluded that there was little evidence of corresponding increases in non-vaccine types among 14-19 year olds. For 20-24 year olds there was an increase in HPV73 only. Finally, Grun et al (the only eligible study which was not an update of a previous publication) considered HPV prevalence in cervical samples collected from young women attending a youth clinic in Sweden. The authors reported declines in the prevalence of HPV16 from 34.7% in 2008-2011 to 18% in 2013-2015. Changes in the prevalence of individual HPV types were presented graphically and there was little evidence of increases between the pre-vaccination and post-vaccination period.

The data that were available from the publications included in this updated review continue to support the finding that there is no clear evidence for type-replacement following introduction of HPV vaccination. Following the publication of the previous systematic review and meta-analysis, it was agreed that the team from Université Laval (Section 3.1) would lead future data collection for both the systematic review on vaccine types and non-vaccine types. Therefore, I did not contact authors for data on changes in individual HPV types because the group at Université Laval will be contacting these authors when data are requested for an updated publication. As most changes in individual HPV types were not included in the four additional publications (or were only presented graphically), the meta-analysis and forest plots were not updated in this thesis.

There were several publications excluded from this updated review either because, (i) the analyses compared HPV prevalence among vaccinated and unvaccinated women within the post-vaccination period[98-112], (ii) the analyses were restricted to measuring HPV infection at a single time point in the post-vaccination period and did not stratify by vaccine status[113], or (iii) the analyses were restricted to comparing HPV prevalence over time within the post-vaccination period[114, 115]. In Section 4.2, I describe the advantages and disadvantages of considering changes within the post-vaccination period only rather than comparing prevalence between the pre- and post-vaccination period. For this analysis, the focus was on assessing relatively small changes in the prevalence of non-vaccine types rather than the larger direct protection of vaccination on vaccine types. Therefore, it was more appropriate to consider the population-level effect rather than limiting comparisons to vaccinated and unvaccinated women which may be affected by inequalities in who is being vaccinated. However, as the time since vaccine introduction increases, there could be an argument that future reviews could include changes in non-vaccine HPV prevalence over time within the post-vaccination period.

Chapter 4: Methods for HPV infection surveillance to evaluate the National HPV Immunisation programme

In this chapter, I describe the methods of repeat cross-sectional surveillance of HPV infection in young sexually active women attending for chlamydia screening which was established to evaluate the HPV Immunisation Programme. I first consider the expected changes in HPV prevalence following the introduction of HPV vaccination and the strengths and limitations of using surveillance data to monitor such changes. I then describe the study population and my role in this surveillance including the data and specimen collection, data linkage and statistical methods to inform research questions 1, 2 and 3 of this thesis (Figure 2.4). I also describe additional methodology that I developed to improve interpretation of changes in HPV prevalence.

4.1. Expected changes in the prevalence of HPV infection following national HPV16/18 vaccination

4.1.1. Reductions in vaccine HPV types

Given the high efficacy of HPV vaccines and the high coverage achieved by the vaccination programme, it was expected that there would be large declines in the HPV vaccine types following the introduction of vaccination. To predict the likely scale of these declines, a simplistic approach would be simply to multiply the vaccine efficacy from the clinical trials with the vaccine coverage in the population. However, the reductions in HPV vaccine-type infections in a population-based surveillance study will likely differ from those seen in the clinical trials for several reasons. Firstly, the proportion of women vaccinated who had a prior HPV infection will vary depending on the population and age at vaccination. As the vaccine has no efficacy against clearance of an existing infection, this will reduce the impact of HPV vaccination. Secondly, there may be some inequalities in who receives vaccination. For example, if vaccinated women are those at a lower risk of infection (either at the

time of vaccination or subsequently) then this could affect the expected population-level impact of the vaccine (the methods and results of surveillance to investigate inequities in HPV vaccination coverage are described in Chapter 7 and Chapter 8). Finally, in a population with high vaccine coverage, we would expect some herd protection among unvaccinated women, which would increase the impact of vaccination compared to results seen in individually randomised pre-licensure clinical trials.

4.1.2. Reductions in phylogenetically related HPV types (cross-protection)

As described in Section 2.6.3, the results from the clinical trials of the bivalent vaccine provided evidence of cross-protection against persistent infection with HPV31, HPV33 and HPV45, three of the high-risk types which are phylogenetically closely related to the HPV vaccine types. There was also some evidence of cross-protection against HPV51 (not included in either the α7 or α9 species group which include the vaccine types; Table 2.1)[73]. Therefore, moderate declines in the cross-protective HPV types were expected. As described in the previous section, the scale of these declines is also dependent on the vaccination coverage, the risk of exposure to HPV prior to vaccination and herd protection effects.

4.1.3. Potential increases in other high-risk HPV types (type-replacement)

With the declines in HPV vaccine types and closely related types, there has been some concern that this could lead to other HPV types filling an ecological niche and taking their place (known as type-replacement). I described this in detail in Chapter 3 along with results of the systematic review to investigate changes in the non-vaccine HPV types following the introduction of national HPV vaccination. Although these data provide no clear evidence for increases in the HPV non-vaccine types to date, it is prudent to remain vigilant for such changes. If type-replacement was to occur, this could have a serious effect on the potential impact of HPV vaccination on the incidence of cervical pre-cancer and cancer. Such information is required to

inform effectiveness and cost-effectiveness models that will inform changes in the HPV vaccination programme (for example, the choice of vaccine used in the national programme). Therefore, if increases in non-vaccine HPV types were seen, it would be imperative to explore, and rule out, other possible reasons for such changes before concluding that this was due to type-replacement.

4.1.4. How to interpret changes in HPV prevalence

One of the challenges of interpreting national surveillance data is to ascertain what are the true effects of HPV vaccination (i.e. effectiveness against the vaccine-types, effectiveness against the closely related HPV types, type-replacement) and what are the effects not related to HPV vaccination (i.e. effects of chance, changes in the surveillance population unrelated to vaccination, the change in the HPV assay over time, unmasking effect).

In Section 4.4 and Chapter 5, I describe the methods that I developed to disentangle the true effects of HPV vaccination from other changes, unrelated to vaccination.

4.2. National surveillance of HPV DNA infection: Aims and background

4.2.1. Aims of national surveillance of HPV infection

As previously described, national HPV infection surveillance is required to monitor the population-level impact of the National HPV Immunisation Programme. There are two distinct approaches to such surveillance, which offer different benefits and challenges.

The first option is to compare the prevalence of HPV infection over time and explore how changes are associated with vaccination coverage. In the simplest example, HPV prevalence in a survey conducted prior to the introduction of HPV vaccination is compared to prevalence in a similar survey conducted following vaccine

introduction. This estimates the population-level changes in HPV infection that incorporates the direct effect of vaccination and indirect effect of herd protection among both vaccinated and unvaccinated individuals. However, this analysis is subject to bias if there are other changes over time that affect HPV infection in the population but are unrelated to HPV vaccination.

The second option is to compare the prevalence of HPV infection in vaccinated women compared to unvaccinated women in the post-vaccination period. This requires data on individuals' vaccination status and allows direct calculation of vaccine effectiveness. However, the effectiveness estimate may be confounded by risk factors for HPV infection that may be inequitably distributed among vaccinated and unvaccinated females. For example, if those being vaccinated are those at lower risk for HPV infection then we would overestimate the true vaccine effectiveness.

Ideally, any surveillance would incorporate both the above options to explore fully the impact of the vaccination programme on HPV infection.

4.2.2. My role in this surveillance

Prior to the start of this PhD, a survey of HPV prevalence had been conducted by PHE in the pre-vaccination period and post-vaccination surveillance had been established.

My role was to lead the PHE evaluation of the HPV Immunisation Programme in England and this, together with extended work, formed this section of this PhD. I continued to lead the collection of samples from the post-vaccination period and I designed and conducted all analyses on changes in the prevalence of HPV infection within the post-vaccination period compared to the pre-vaccination period.

Prior to the start of this PhD, there was also an intention to collect HPV vaccination status on women included in this surveillance. This was more complex than initially expected given the lack of a single national immunisation database. Therefore, as part of my role at PHE and forming part of this PhD, I established methods and conducted data collection of HPV vaccination status for a subset of women included in the post-vaccination surveillance (Section 4.3.6). This allowed two important additional analyses. Firstly, as described above, this enabled direct calculation of the vaccine effectiveness in this population. Secondly, I compared the prevalence of HPV vaccine-types among unvaccinated women in the post-vaccination period with the prevalence among women in the pre-vaccination period to provide an estimate of the herd protection effect. I also describe work I devised and conducted as part of this PhD to adjust for the change in assay over time when comparing HPV prevalence between the pre- and post-vaccination periods (Section 4.4).

My role in this surveillance is further clarified in the cover sheets for the publications in Chapter 6 of the thesis.

4.2.3. Source of routine samples for HPV testing

It was announced in 2016 that HPV primary screening would be introduced in England (Section 2.5). However, cervical screening in England does not start until age 25 years old and primary HPV screening had yet to be introduced at the time of this PhD. Therefore, it was necessary to find a source of routinely collected residual samples originally obtained for other purposes that could be tested for HPV infection. These samples should ideally be collected from younger sexually active women to be able to demonstrate an effect of HPV vaccination as early as possible. Such samples also needed to be suitable to identify a current HPV DNA infection. Urine specimens lack sensitivity to detect HPV infection and swabs taken from the cervix or vagina are more suitable to determine a current HPV DNA infection with high sensitivity[116].

As cervical screening is not offered in England until young women reach the age of 25 years, the first cohort of vaccinated women would not attend for cervical screening for at least seven years following the introduction of HPV vaccination. To obtain an alternative source of cervical or vaginal swabs taken from younger, sexually active women, PHE made use of residual samples collected from young women attending for chlamydia screening using a vulva-vaginal swab (VVS) specimen (Section 4.2.4).

4.2.4. The National Chlamydia Screening Programme (NCSP)

Chlamydia is the most commonly diagnosed bacterial sexually transmitted infection in England and is particularly prevalent among young men and women[50]. The majority of chlamydia infections are asymptomatic hence most infected individuals will be unaware of their infection. However, if left untreated, chlamydia infection can lead to pelvic inflammatory disease (PID) which can in turn lead to long-term pelvic pain, infertility and ectopic pregnancy. Consequently, chlamydia screening in England is recommended annually and on sexual partner change for all sexually active men and women aged 24 years old and under[117] in order to control chlamydia infection and disease sequelae.

In England, there was a phased introduction of the National Chlamydia Screening Programme (NCSP) from 2003 to 2008. Chlamydia screening is offered opportunistically when eligible individuals attend a range of different venues (including both clinical and non-clinical settings).

Since the introduction of the NCSP, systems were established by PHE to collect individual-level data from chlamydia tests carried out through the programme. More recently, this has been expanded to collect data on all chlamydia tests undertaken in England at NHS laboratories, local authorities and NHS-commissioned laboratories using the Chlamydia Testing Activity Dataset (CTAD) (described in

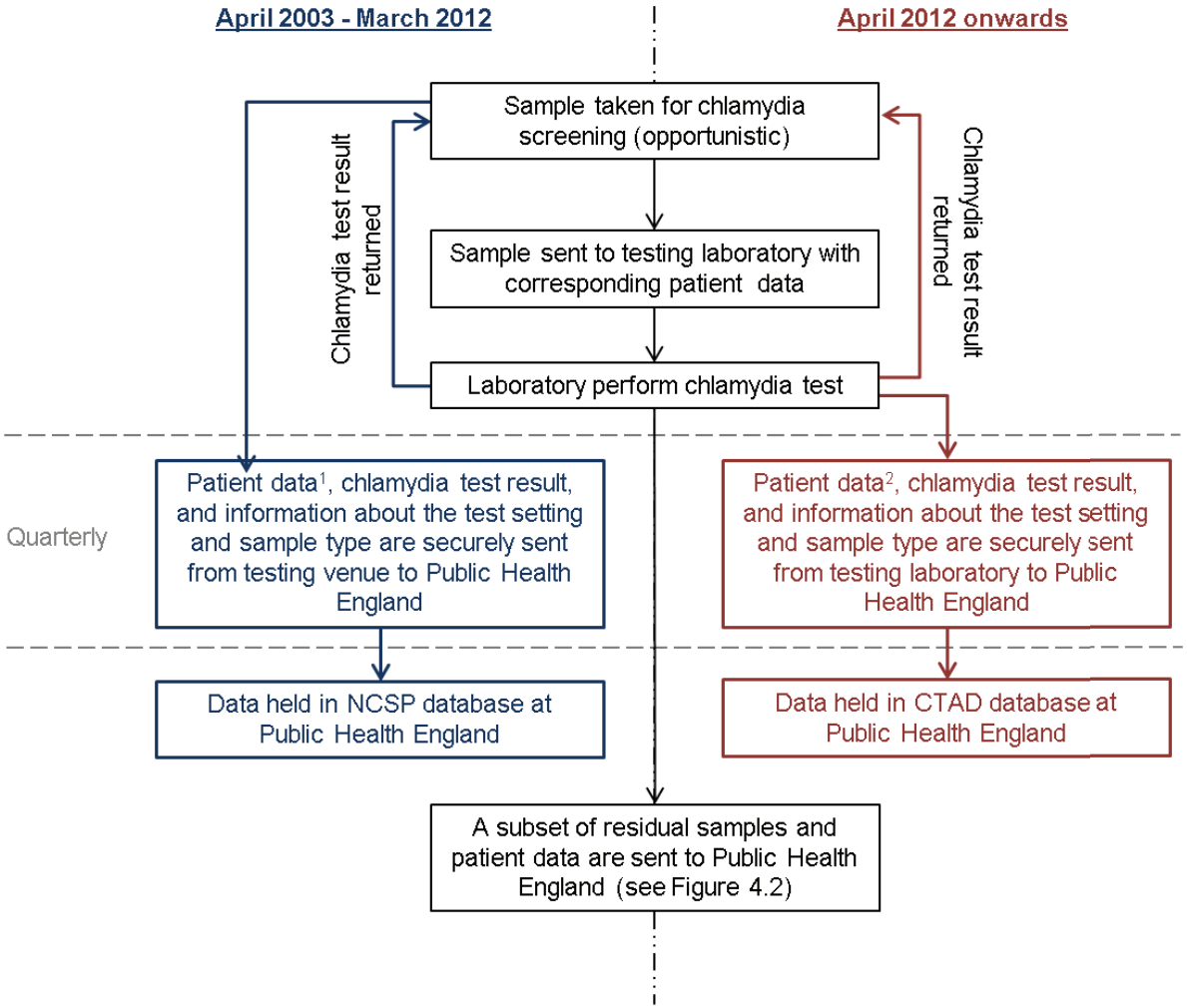
Section 4.2.5). Data from 2016 reported that over 1.4 million chlamydia tests were carried out in 15-24 year olds in England (representing around 30% of females and 12% males in the target population tested if we assume one test per person per year) with 128,098 chlamydia diagnoses [118]. These samples are usually self-taken[119]. Patient instructions vary slightly by area and collection device but for women, the sample is generally collected by inserting the swab around two inches into the vagina and gently rotating for between 10 and 30 seconds. The patient is then instructed to insert the swab into a tube and seal this before either returning to the health practitioner or returning directly to the testing laboratory.

4.2.5. Data collection for the NCSP

Between 2003 and March 2012, all data on chlamydia tests and diagnoses conducted as part of the NCSP were compiled to produce the NCSP dataset. These data were submitted to PHE quarterly from all testing venues participating in the NCSP. Data collected included patient sociodemographic data, sexual behaviour data and information about the chlamydia test performed (Figure 4.1 and Table 4.1).

In April 2012, there was a substantial change to the way that chlamydia testing data were reported. The introduction of the Chlamydia Testing Activity Dataset (CTAD) meant that data were submitted directly from the testing laboratories rather than the testing venues where patients were seen (Figure 4.1). Each laboratory reports individual-level data, based on the information provided from the clinic, when the sample is sent for testing. These data are submitted quarterly but some of the data collected differs from the NCSP dataset. As before, data are collected on sociodemographics and the chlamydia test result although there are some differences in the data completeness (for example, ethnicity is more often missing in CTAD). However, CTAD collects no data on sexual behaviour and does not record the reason for the test. Full details of the comparison between the two datasets are provided in Table 4.1.

Figure 4.1: Collection of data from women undergoing chlamydia screening in England since 2003



1 See NCSP data fields in Table 4.1

2 See CTAD data fields in Table 4.1

Table 4.1: Data fields available in the National Chlamydia Screening Programme (NCSP) database and Chlamydia Testing Activity Dataset (CTAD)

Data field	NCSP database (2003 - March 2012)	CTAD (April 2012 - date)
Identifiers		
Patient identifier (NCSP/CTAD)	Data completeness 98%	Data completeness 91%
NHS number	Data completeness 8%	Data completeness 30%
Socio-demographic data		
Date of birth	Data completeness 100%	Data completeness 100%
Gender	Data completeness 100% 1 Male 2 Female	Data completeness 100% 1 Male 2 Female 9 Indeterminate
Ethnicity	Data completeness 73% Patient ethnicity based on ONS classification[1]	Data completeness 19% Patient ethnicity based on ONS classification[1]
Postcode of residence	Data completeness 88%[2]	Data completeness 60%
Postcode of GP	Not collected	Data completeness 47%
Postcode of testing service	Not collected	Data completeness 90%
Sexual behaviour data		
Did the patient report two or more sexual partners in previous 12 months?	Data completeness 54% 1 Yes 2 No	Not collected
Did the patient report a new sex partner in the last 3 months?	Data completeness 56% 1 Yes 2 No	Not collected
Details of chlamydia test		
Reason for test	Data completeness 99% A03 Chlamydia screening A04 Diagnostic testing A08 Contact of chlamydia positive A11 Reports symptoms of STI	Not collected
Testing service type	Data completeness 100% 01 Gynaecology and fertility 02 Outreach 03 Antenatal 04 Occupational Health 05 Pharmacy 06 Community Sexual Health Services 07 General Practice 08 Accident and Emergency 09 Remote testing 10 Military 11 Prisons and YOI 12 Chlamydia Screening Office 13 Termination of Pregnancy 14 Education 15 Youth	Data completeness 99% 01 GUM services 02 Community Sexual Health Services 3 General Medicine Practitioner Practice 04 Pharmacy Premises 05 TOP Services 06 Internet XX Other

Specimen type	Data completeness 99% 1 Urine sample 2 Cervical swab 3 Vulva/vaginal swab 4 Urethral swab 5 Other	Data completeness 100% 1 Urine 2 Genital 3 Rectal 4 Pharyngeal
Specimen date	Data completeness 100%	Data completeness 98%
Result of chlamydia test	Data completeness 100% 1 Positive 2 Negative 3 Equivocal 4 Insufficient Specimen 5 Inhibitory result	Data completeness 100% 01 Positive 02 Negative 03 Equivocal 04 Insufficient Specimen 05 Inhibitory result XX Other

1: Ethnicity in CTAD categorised as: White: British, Irish, any other White background; Mixed: White and Black Caribbean, White and Black African, White and Asian, Any other mixed background; Asian: Indian, Pakistani, Bangladeshi, any other Asian background; Black or Black British; Caribbean, African, any other Black background; Other Ethnic Groups: Chinese, Any other ethnic group, not stated.
2: Postcode of residence was available but was not linked prior to anonymisation for pre-vaccination surveillance. Therefore IMD could not be calculated in the pre-vaccination period and was only available in the post-vaccination period (see Section 4.3.12).

4.3. National surveillance of HPV DNA infection: Methods

4.3.1. HPV surveillance study population

A small subset of laboratories conducting chlamydia screening tests was selected to participate in the national HPV infection surveillance. Prior to the start of this PhD, laboratories were recruited from across the country and from a mix of urban and rural areas. The recruitment of these laboratories was also based on their throughput of eligible specimens (at least 700 specimens over a 6-month period).

PHE requested that laboratories identify a target number of residual VVS samples from eligible women (based on information provided from the testing venue) for the purposes of national HPV infection surveillance. Eligible women were defined as those;

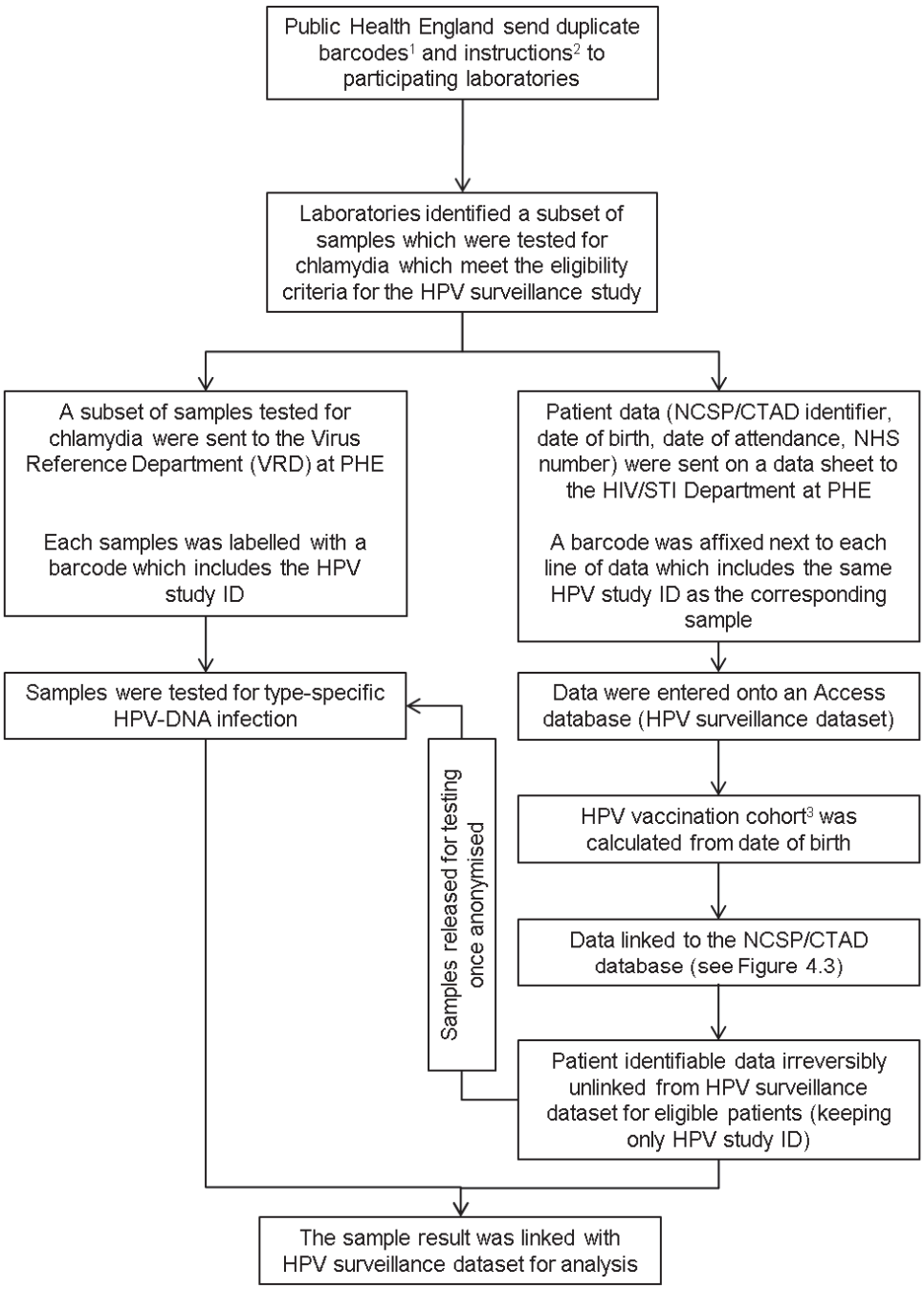
- Aged 16 to 24 years old
- Having a sample taken for opportunistic chlamydia screening (i.e. not a symptomatic test or partner notification test). This criterion was to exclude

those with a far higher risk of chlamydia who were also likely to have a higher risk of HPV infection.

- Being screened at either a General Practice, a Youth Clinic or Sexual and Reproductive Health Clinics (also known as Community Sexual Health Services, Contraceptive Sexual Health Services or Family Planning Clinics). This criterion was applied to maintain a more stable population over time that was less subject to bias from changes in attendance patterns at other settings.

The national HPV surveillance was established at six testing laboratories in 2008 for the pre-vaccination period. The collection of post-vaccination period specimens was established at 10 testing laboratories in 2010 (the same six laboratories as the pre-vaccination period as well as an additional four laboratories in order to increase the number of samples collected in the post-vaccination period). The process of data and sample collection and data linkage are shown in Figure 4.2 and described in detail in Sections 4.3.3 to 4.3.5. These processes were in place prior to my involvement with this surveillance. As highlighted in Section 4.2.2, I have been the lead on this surveillance since October 2011, and I have conducted (or overseen) all these processes. The pre-vaccination prevalence data were published prior to the start of this PhD but I designed and conducted all analyses of post-vaccination data. Sections 4.3.6, 4.3.7 and 4.3.10 to 4.3.17 describe the methods that I established for this surveillance.

Figure 4.2: Methods to request and collect residual specimens from 10 chlamydia testing laboratories across England who participated in the HPV surveillance study



1: Two barcodes were sent for each unique study number. One of these barcodes was attached to the sample and the other to the data sheet

2: I periodically sent instructions to laboratories which included eligibility criteria, target number of samples, how to aliquot, label and send samples and how to send corresponding data. Laboratories were asked not to select samples based on chlamydia test result.

3: HPV vaccination cohorts were based on school years with routine vaccination offered to 12-13 year old girls and catch-up vaccination offered to girls ages up to 18 years old.

4.3.2. Ethics

This surveillance to monitor the impact of HPV vaccination using residual chlamydia samples was initially reviewed and approved by the South East Research Ethics Committee (REC reference: 10/H1102/7). Patient identifiable data were used to assess eligibility and also to enable linkage with chlamydia results and HPV vaccination status. Prior to HPV testing, all patient identifiable data were irreversibly deleted. Individual patient consent was not required as this study tested these anonymised specimens (with patient-identifiable data deleted prior to testing) as part of Public Health Surveillance conducted to monitor the HPV vaccination programme.

In September 2014, this surveillance was reclassified as Public Health Monitoring as part of PHE's national remit and therefore was withdrawn from the Research Ethics Committee.

4.3.3. Collection of residual samples

Pre-vaccination era specimens were collected between January 2008 and September 2008, prior to the introduction of HPV vaccination. Post-vaccination era specimen collection started in October 2010 and this PhD includes results from samples collected up to December 2016. All women included in these analyses will have been offered the HPV16/18 vaccine as part of the National HPV Immunisation Programme.

Sample collection for the pre- and post-vaccination surveillance was identical. Each year of the surveillance, The HPV surveillance team at PHE (either me or overseen by me) sent updated instructions to each laboratory to request the target number of samples from each age-group. Laboratories were asked to send the following to the Virus Reference Department (VRD) at PHE:

- A minimum of 2ml of residual sample after the chlamydia test had been performed refrigerated (not frozen) prior to sending.
- Anonymised sample tubes labelled only with a unique barcode (duplicate barcode labels were provided by PHE). All other patient identifiers removed from the sample tubes prior to sending.

Chlamydia testing laboratories were asked not to select samples based on the chlamydia test result, in order to collect a representative sample of chlamydia negative and chlamydia positive specimens.

4.3.4. Data collection for residual samples

Along with the specimen, the laboratories were also asked to complete a data collection form (blank versions were provided by PHE). Chlamydia testing laboratories were asked to attach the duplicate of the barcode that was attached to the residual sample alongside the corresponding patient information, which included:

- The date of sample collection
- The date the sample was sent to VRD
- Patient's date of birth
- NCSP/CTAD identifier
- NHS number, when available

These data collection forms were sent securely to the HPV surveillance team at Public Health England (separately from the sample tubes). Data from the data collection forms were entered onto a secure Access database designed specifically for this surveillance study. Data were entered by two separate individuals and any discrepancies were checked with the original list. Any obvious errors on data

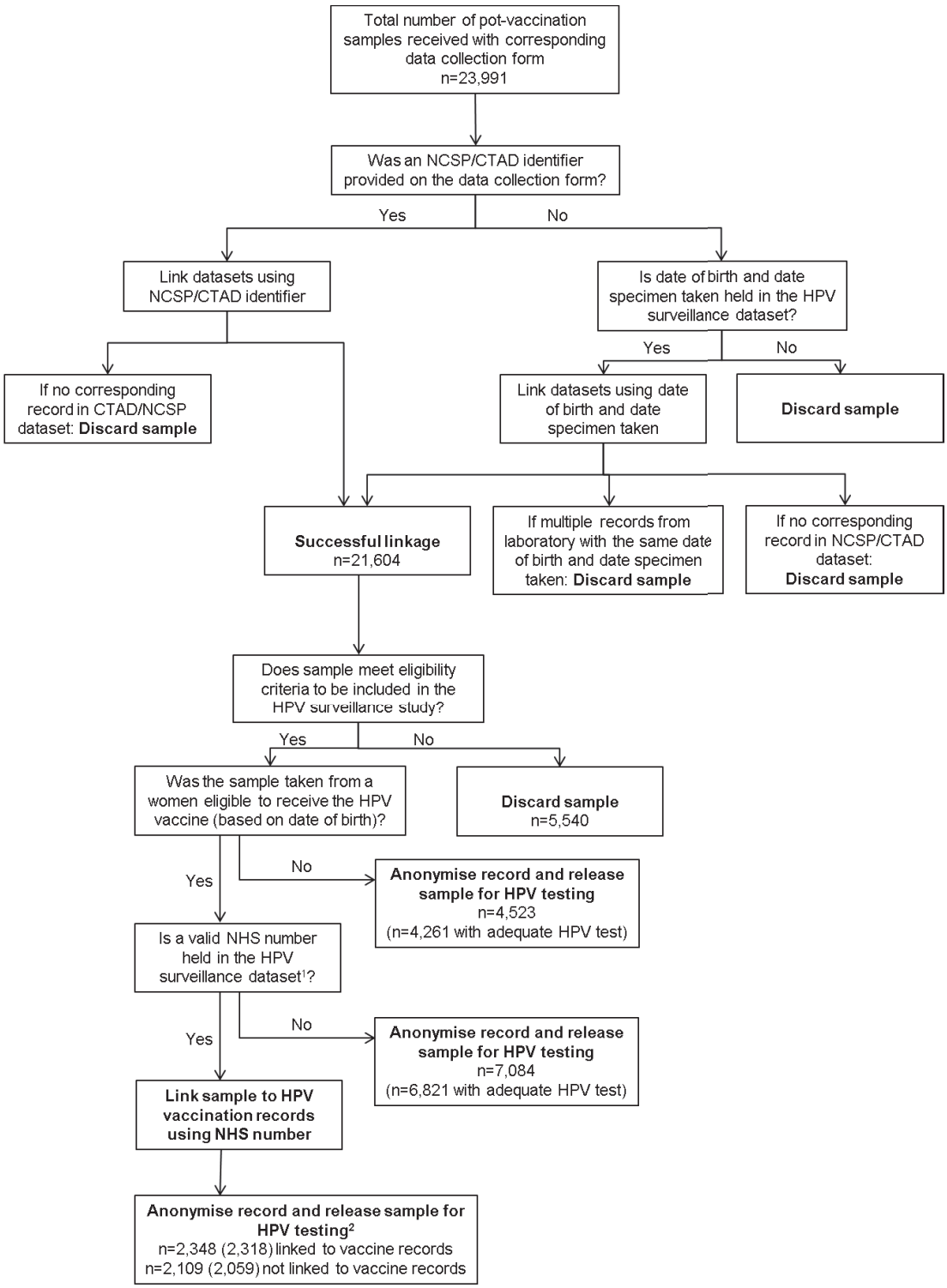
collection forms (e.g. dates in the future or 8-digit NHS numbers) were checked with the chlamydia testing laboratories.

4.3.5. Linkage of HPV surveillance data to the NCSP database and CTAD

Using Stata version 12 (StataCorp LP), I linked the HPV surveillance data with the NCSP database or CTAD database depending on the year of sample collection. This linkage was performed to confirm eligibility of samples (as outlined in Section 4.3.1) as well as obtain some additional information reported at the time of the chlamydia screen (as described in Table 4.1).

The patient identifiers included on the data collection forms were used for linkage. When possible, I used the NCSP/CTAD identifier that uniquely identifies an individual chlamydia test. However, for some laboratories, this identifier was not available and therefore could not be provided on the data collection forms. If NCSP/CTAD identifiers were unavailable, the patient's date of birth and the date of the specimen were used to link to the relevant laboratory's data. Ten percent of residual samples could not be matched to NCSP/CTAD data suggesting that; (i) the testing venue and/or laboratory had not submitted this chlamydia test to the NCSP database or CTAD in error, (ii) incorrect data had been submitted to the NCSP database or CTAD, or, (iii) incorrect data had been entered to the data collection form for the HPV surveillance. These samples were not released for HPV testing. Further details of this linkage are provided in Figure 4.3.

Figure 4.3: Algorithm to link the NCSP/CTAD dataset with the HPV surveillance specimens and data



1: *Either from the data collection form or from NCSP/CTAD dataset*
2: *The reason that some records were not linked to vaccination status was either because the local CHIS system was unable to perform the linkage or the CHIS system could not identify an individual's record. Numbers in brackets are the numbers with an adequate HPV test.*

For samples which were linked using an NCSP/CTAD identifier, I verified that the date the sample was taken and date of birth in the NCSP/CTAD database matched the data on the data collection forms.

I calculated the age of the women at testing using their date of birth and the date the specimen was taken. Ineligible samples were identified using the eligibility criteria given in Section 4.3.1, specifically;

- Out of eligible age-range for the surveillance
- Invalid reason for test (as recorded in NCSP dataset – not available in CTAD dataset)
- Invalid venue type (as recorded in NCSP/CTAD dataset)
- Invalid specimen type (as recorded in NCSP/CTAD dataset)
- Sample not taken from a female (as recorded in NCSP/CTAD dataset)

Twenty-one percent of samples were ineligible according to the above criteria and were not released for HPV testing.

4.3.6. Linkage of HPV surveillance data to HPV vaccination records

As part of the national HPV surveillance, I aimed to obtain retrospectively the HPV vaccination status for all eligible women in the post-vaccination period. Briefly, vaccination records were collected using two approaches:

1. Collected directly from the chlamydia testing laboratory using the chlamydia-test request form (based on self-reported vaccination status)
2. I contacted the Child Health Record Departments for each relevant local authority (this comprised between 1 and 7 local authorities for each testing laboratory). I requested HPV vaccination records for eligible women, defined as women included in the HPV surveillance, who had:
    - a valid NHS number

- a known local authority of residence (based on postcode)
- a date of birth on or after 1st September 1990 (i.e. those who would have been eligible to receive the HPV vaccine as part of the National HPV vaccination Programme)

On receipt, I linked these HPV vaccination data to the HPV infection surveillance database to allow further analyses of the post-vaccination data (see Sections 4.2.1 and 4.3.15). I describe the methods to collect HPV vaccination records from Child Health Records Department in further detail in Section 5.2.

4.3.7. Estimated vaccination coverage

As described in the Background (Section 2.7.2), national HPV vaccination data are collated and published by PHE, using local area-level data collected using the ImmForm website.

For those women for whom it was not possible to link to HPV vaccination status (Section 4.3.6), I estimated HPV vaccination coverage using these nationally reported data to analyse the association between HPV vaccination coverage and changes in HPV prevalence (as described in Section 4.3.14). Specifically, I attributed the national reported vaccination coverage data for the relevant birth cohort to each individual record, summed these and divided by the total number of records (i.e. a weighted average). An example is given in Table 4.2.

Table 4.2: An example calculation of estimated HPV vaccination coverage in the HPV surveillance population

Patient	Vaccination status	National coverage[1]	Individual contribution
1	Vaccinated	0.784	1
2	Unvaccinated	0.784	0
3	Unknown	0.784	0.784
4	Vaccinated	0.784	1
5	Unknown	0.784	0.784
6	Unknown	0.784	0.784
7	Unknown	0.784	0.784
8	Vaccinated	0.784	1
9	Vaccinated	0.784	1
Sum			7.136
Estimated coverage (sum/total number of patients)			0.793

1: for relevant birth cohort

The vaccinated birth cohorts by date of birth are given in Table 4.3 with the national coverage. I calculated 1-dose and 3-dose coverage which included vaccination in the year the vaccine was offered and mop-up vaccination (i.e. vaccine doses given to girls who either started or completed their vaccination late). Women recorded as vaccinated but with an unknown number of doses were assumed to be fully vaccinated in the main analysis; this was considered further in the sensitivity analysis (Section 6.3).

4.3.8. Anonymisation of HPV surveillance data

Following linkage of the HPV surveillance data with the NCSP/CTAD databases (and HPV vaccination data for relevant specimens), I irreversibly deleted all personal identifiable patient data prior to releasing samples for HPV testing (including all identifiable data collected on the data collection forms and held in the Access database), keeping the barcode number. Therefore, it was not possible, at any stage to link HPV infection result back to an individual.

Table 4.3: Vaccination cohorts of eligible women included in HPV infection surveillance

Year HPV vaccination first offered	Date of birth	Vaccination cohort	Age first offered HPV vaccination	National vaccination coverage[1]		
				1 or more doses (%)	2 or more doses (%)	All 3 doses (%)
2008/09	01/09/1995 - 31/08/1996	Routine	12-13 years old	89.4	87.7	84.4
2008/09	01/09/1990 - 31/08/1991	Catch-up	17-18 years old	66.1	59.3	47.4
2009/10	01/09/1996 - 31/08/1997	Routine	12-13 years old	85.9	84.1	80.9
2009/10	01/09/1994 - 31/08/1995	Catch-up	14-15 years old	81.9	79.6	75.7
2009/10	01/09/1993 - 31/08/1994	Catch-up	15-16 years old	78.4	75.8	70.8
2009/10	01/09/1992 - 31/08/1993	Catch-up	16-17 years old	59.8	55.9	48.1
2009/10	01/09/1991 - 31/08/1992	Catch-up	17-18 years old	55.6	50.3	38.9
2010/11	01/09/1997 - 31/08/1998	Routine	12-13 years old	88.9	87.5	84.2
2011/12	01/09/1998 - 31/08/1999	Routine	12-13 years old	90.6	89.6	86.8
2012/13	01/09/1999 - 31/08/2000	Routine	12-13 years old	90.9	89.6	86.1
2013/14	01/09/2000 - 31/08/2001	Routine	12-13 years old	91.1	89.8	86.7
2014/15	01/09/2001 - 31/08/2002	Routine	12-13 years old	89.4	85.1[2]	
2015/16	01/09/2002 - 31/08/2003	Routine	12-13 years old	87.0	83.1[2]	
2016/17	01/09/2003 - 31/08/2004	Routine	12-13 years old	87.2	Pending[2]	

1: Includes mop-up vaccination performed after the year vaccination was first offered

2: The National HPV Immunisation Programme moved to a 2-dose schedule from September 2014. In some local areas, vaccine doses were given 12 months apart. Data are collated and published annually hence data on full 2-dose coverage are not available until 2 years after the first dose is given

4.3.9. HPV testing

The assay used for HPV testing changed between the pre- and post-vaccination periods.

Specimens collected in the pre-vaccination period were initially tested by the Hybrid Capture 2 (HC2) HPV DNA test, using the Combined Probe Cocktail Method to detect high-risk and possible high-risk types (as above) and five low-risk types (6, 11, 42, 43 and 44). Relative light units/cut off (RLU/CO) greater than 1 were considered positive. Samples which were HC2 positive were then genotyped by the Linear Array HPV Genotyping test (Roche Molecular Systems). DNA was extracted using the automated BioRobot Universal platform (Qiagen, UK) and then amplified using PGMY primers. The LA test identified 37 HPV types (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73 (MM9), 81, 82 (MM4), 83 (MM7), 84 (MM8), IS39, and CP6108)

Specimens collected in the post-vaccination period were tested for type-specific HPV DNA to detect 13 high-risk types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68), five possible high-risk types (HPV26, 53, 70, 73 and 82) and two low-risk types (HPV6 and 11), using an in-house multiplexed PCR and Luminex-based genotyping test. DNA was extracted from residual VVS for use in a multiplexed, block-based PCR with HPV-specific oligonucleotides and two `housekeeping' gene oligonucleotides. The result was reported as inadequate if the samples were negative for both HPV and the housekeeping gene, pyruvate dehydrogenase (PDH).

All the high-risk HPV types which were detectable by the pre-vaccination test were also included in the post-vaccination test. However, there may well have been differences in the detection rates of the two testing methods. A validation study was conducted by the VRD to compare the two tests and I describe this, along with the

potential implications of the assay change, in more detail in Section 4.4 and Chapter 6.

The following Sections (4.3.10 to 4.3.17) describe the data analysis which I designed and conducted to address research questions 1, 2 and 3 of this thesis (Figure 2.4).

4.3.10. Data coding and descriptive analysis

I linked HPV test results with the anonymised HPV surveillance dataset, using the unique barcode number.

Samples with an inadequate HPV result were excluded from the analysis. HPV prevalence was calculated for each specific HPV type. I also calculated combined HPV prevalence, restricted to types included in both the pre-vaccination and post-vaccination assays, for (i) any high-risk HPV type; (ii) the HPV vaccine types (HPV16 and/or 18); (iii) the additional high-risk HPV types included in the nonavalent HPV vaccine (HPV31, 33, 45, 52 and/or 58); (iv) the HPV types for which there is evidence of cross-protection from clinical trials: HPV31, 33 and/or 45 (HPV51 was not included here as the evidence of potential cross-protection against this type was not consistent in the clinical trial; see Sections 2.6.3 and 4.1.2) and (v) the non-vaccine high-risk HPV types (i.e. any high-risk type not including HPV16 or 18).

I divided the post-vaccination period into separate time periods with broadly similar time since vaccination, vaccination coverage and age at vaccination; 2010–2011, 2012-2013, 2014-2015 and 2016.

I categorised ethnicity as white (including white, white British, white Irish and any other white background), black (including black or black British, black Caribbean, black African, white and black Caribbean, white and black African and any other

black background), Asian (including Asian or Asian British, white and Asian, Indian, Pakistani, Bangladeshi and any other Asian background) and Other (including Chinese, any other mixed background and any other ethnic group). The chlamydia test results were recorded as positive, negative or unknown (if there was an inconclusive result).

I compared differences in ethnicity, sexual behaviour, sample collection venue, and chlamydia test results between the pre- and post-vaccination period and over time within the post-vaccination period to explore whether there were changes in the study population attending for chlamydia screening. HPV prevalence was calculated for each individual HPV type and groups, as defined above.

4.3.11. Stratification of results by age at sample and age at vaccination

All analyses comparing HPV prevalence over time (see Sections 4.3.12 to 4.3.14) were stratified by three age-groups, denoting the age at which the sample was taken (16-18 years old, 19-21 years old and 22-24 years old). As previously described (Section 2.3), HPV prevalence varies by age, hence considering changes in HPV prevalence in a specific age-group provided a more stable population for comparison over time with differences more likely to be due to HPV vaccination.

Table 4.4 describes the association between age at sample collection, year of sample collection and age at vaccination. Age at vaccination is closely related to both vaccination coverage (coverage is higher at younger ages) and the expected effectiveness of the vaccine, largely due to a higher risk of exposure to HPV before vaccination in those vaccinated at older ages (and hence lower vaccine effectiveness). Therefore, analyses of vaccine effectiveness (described in Section 4.3.15) were stratified by the age that vaccination would have been offered as part of the national programme (offered vaccination at 12-15 years old vs. offered vaccination at 16-17 years old).

Table 4.4: Age at which HPV vaccination would have been offered as part of the National HPV Immunisation Programme, by age at sample collection and year of sample collection

Age at sample collection (years)	Age first offered vaccination							
	Pre-vaccination period	Post-vaccination period (by year)						
	2008	2010	2011	2012	2013	2014	2015	2016
16	All samples collected prior to HPV vaccination	14-16	12-15	12-14	12	12	12	12
17		15-17	14-16	12-15	12-14	12	12	12
18		16-17	15-17	14-16	12-15	12-14	12	12
19		17	16-17	15-17	14-16	12-15	12-14	12
20		17	17	16-17	15-17	14-16	12-15	12-14
21		NA[1]	17	17	16-17	15-17	14-16	12-15
22		NA[1]	NA[1]	17	17	16-17	15-17	14-16
23		NA[1]	NA[1]	NA[1]	17	17	16-17	15-17
24		NA[1]	NA[1]	NA[1]	NA[1]	17	17	16-17

1: *Women born before 1st September 1990 were not eligible to receive the HPV vaccine in the national programme*

4.3.12. Comparison of HPV prevalence between the pre- and post-vaccination period

These analyses were conducted on samples collected between 2008 and April 2013.

Changes in the prevalence of HPV between the pre-vaccination and post-vaccination periods were compared using prevalence ratios (PRs), calculated using a log binomial regression model in Stata. The corresponding 95% confidence intervals were calculated using a Wald test. Trends over time were assessed by including the three time periods (pre-vaccination, 2–3 years post-vaccination, 4–5 years post-vaccination) as an ordered categorical variable. I similarly calculated odds ratios (ORs) using a logistic regression model in Stata. The reason for calculating odds ratios in addition to prevalence ratios is described in Section 4.4 along with further detail regarding the adjustment for the change in assay between the pre- and post-vaccination periods.

Data on sexual behaviour were only collected for samples reported through the NCSP dataset and not for samples reported via CTAD (see Section 4.2.5). In the NCSP dataset, there was a high proportion of missing data for this variable (~20% of sexual behaviour data were missing in the pre-vaccination period and >50% in the post-vaccination period). Furthermore, ethnicity was poorly recorded in both the NCSP and CTAD dataset (although there were more missing data in CTAD) and this varied by area and year of data collection. Given the very high proportion of missingness for sexual behaviour data and ethnicity, it was not appropriate to perform any multiple imputation methods[120]. Thus, these variables were not included in the multivariable regression; although they were included in descriptive analyses but should be interpreted with caution. Furthermore, IMD was only available in the post-vaccination period as patient postcode was not linked in the pre-vaccination period and hence IMD was also not included in this multivariable

regression model. Therefore, there were relatively few variables which could be considered for multivariable regression analysis: testing venue type, age and chlamydia test result which was used as a proxy for high-risk sexual behaviour. For both PRs and ORs, all variables were kept in the model.

4.3.13. Sensitivity analyses for comparison of HPV prevalence between the pre- vs post-vaccination period

A further three sub-analyses were conducted using the above approach, as follows:

Analyses restricted to HPV positive samples: Due to the limited number of demographic variables for inclusion in the multi-variable regression model and to go some way towards addressing concerns about changes in prevalence of HPV over time which were unrelated to HPV vaccination (e.g. changes in sexual behaviour which are not fully addressed by adjustment of chlamydia result), I also analysed data restricted to specimens with at least one HR-HPV type detected. This enabled me to consider changes in the relative, rather than absolute, prevalence of specific HPV types. As an example, if 20% of pre-vaccination specimens were high-risk HPV positive (10% of which were HPV31 positive and 10% HPV33 positive), and 30% of post-vaccination specimens were high-risk HPV positive (15% HPV31 positive and 15% HPV33 positive), then there would be a 50% absolute increase for both HPV types. However, there would be no change in the relative frequency of either HPV type among those who were high-risk HPV positive.

Analyses restricted to laboratories participating in both the pre-vaccination and post-vaccination period: The laboratories collecting samples in the pre-vaccination period were also included in the post-vaccination specimen collection to obtain as stable population as possible over time. However, as previously explained in Section 4.3.1, it was necessary to increase the number of collecting laboratories in the post-vaccination period. Therefore, I performed a sub-analysis including only the six laboratories included in both the pre- and post-vaccination periods.

Analyses excluding Lewisham and Leeds laboratories: The chlamydia positivity from the Lewisham and Leeds laboratories was higher than that seen at other laboratories (and higher than expected in these areas compared to the overall chlamydia positivity from these laboratories). The reason for this was explored with the two laboratories but could not be determined. Therefore, there was some concern that samples were selected according to their chlamydia result contrary to instructions from PHE. As a result, I conducted a sub-analysis excluding data from these two laboratories (both laboratories only provided samples for the post-vaccination period).

The results of these sensitivity analyses are shown in Chapter 6.

4.3.14. Comparison of HPV prevalence within the post-vaccination period

These analyses were conducted on post-vaccination samples collected between 2010 and 2016.

I calculated HPV prevalence and 95% confidence intervals for each time period separately. Changes in prevalence over time were compared using a log-binomial regression model with year of data collection as a continuous variable. P-values for trend across years were calculated using the Wald test. As with the pre- and post-vaccination period analysis, multivariable regression models were adjusted for age, testing venue and chlamydia positivity.

To explore the association between changes in the prevalence of HPV infection and estimated HPV vaccination coverage, I included a continuous variable in the regression model with the estimated HPV vaccination coverage (as a proportion) for each combination of year and age-group. This allowed calculation of an adjusted HPV prevalence ratio comparing a female population with no vaccination (i.e. coverage=0) with a fully vaccinated female population (i.e. coverage=1). These analyses were stratified by age-group.

4.3.15. Calculation of vaccine effectiveness

These analyses were conducted on post-vaccination samples with a known vaccination status collected between 2010 and 2016.

For the subgroup of women for whom HPV vaccination status was available (Section 4.3.6), direct comparison of HPV prevalence in vaccinated compared to unvaccinated women was calculated as follows:

$$\textit{vaccine effectiveness} = \frac{\textit{HPV prevalence}_{\textit{unvaccinated}} - \textit{HPV prevalence}_{\textit{vaccinated}}}{\textit{HPV prevalence}_{\textit{unvaccinated}}}$$

Results were stratified by age-group (16-18 years old, 19-21 years old and 22-24 years old). Vaccine effectiveness against HPV prevalence was assessed using a log binomial regression model in Stata. A multivariable regression model was used to adjust the vaccine effectiveness estimates for testing venue type, age and chlamydia positivity which was used as a proxy for high-risk sexual behaviour (as in Section 4.3.12). The vaccine effectiveness was calculated as 1-aRR. As a sensitivity analysis, I also adjusted for patients' IMD, available in the post-vaccination period only in a complete case analysis; the results of this are presented in Section 6.4.3.

4.3.16. Sensitivity analyses for comparison of HPV prevalence in the post-vaccination period and for calculation of vaccine effectiveness

In the post-vaccination period only, data on index of multiple deprivation (IMD) were available for some patients. The initial intention was to adjust for IMD as an additional potential confounder, but due to varying proportions of missing data over time, this was not carried out for the main analyses. However, as a sensitivity analysis, I conducted a complete case analysis comparing the unadjusted prevalence ratio for HPV associated with estimated HPV vaccination coverage and the adjusted prevalence ratio (with and without further adjustment for patients' quintile of IMD). I also adjusted the vaccine effectiveness for patients' IMD in a complete case analysis. The results of both of these sensitivity analyses are provided in Section 6.4.3.

Furthermore, prior to 2014, the recommended dose schedule for the bivalent vaccine was three doses, with the second dose given between 1 and 2.5 months after the first dose and the third dose between 5 and 12 months after the first dose. However, if the second or third dose was not administered within the recommended timeframe, the advice is that the course should still be continued without repeating previous doses[121]. In sensitivity analyses, I recalculated the vaccine effectiveness including only women who were known to have received the full vaccine course within the recommended time interval (i.e. excluding those receiving less than three doses, those receiving an unknown number of doses or those who received three doses but outside of the recommended time interval).

4.3.17. Estimation of the herd protection effect of vaccination

To estimate the herd protection effect, I compared the HPV prevalence in the pre-vaccination period to the HPV prevalence in unvaccinated women in the post-vaccination period. As above, results were stratified by age-group (16-18 years old, 19-21 years old and 22-24 years old). Similar to methods described in Section

4.3.12, differences in the prevalence between the unvaccinated and vaccinated women were compared using PRs, calculated using a log binomial model regression model in Stata. A multivariable regression model was used to compare estimates, adjusted (as with other analyses) for testing venue type, age and chlamydia test result to adjust for known changes over time. Odds ratios were adjusted for the change in assay between the pre- and post-vaccination periods as described in the next section.

4.4. Adjustment to assess and correct for differences in HPV detection between pre- and post-vaccination periods

4.4.1. Comparison of pre- and post-vaccination HPV test detection rate

As previously described, in this national surveillance, the assay used to test residual samples for HPV DNA infection in the pre-vaccination period was different to the assay used in the post-vaccination period. The decision to change assay was largely due to the reduced cost of performing relatively high-throughput HPV testing with an in-house assay rather than a commercial assay.

Therefore, whilst the HPV assay used to test specimens has been constant throughout the post-vaccination period, this is different from the assay used in the pre-vaccination period. Given this change in HPV assay between the two periods, changes in the prevalence of specific HPV types may not be due to HPV vaccination alone but could be affected by the different detection rates of these two HPV assays.

4.4.2. Validation study comparing pre- and post-vaccination HPV assays

This validation study was conducted by the VRD at PHE and does not constitute part of this PhD.

Prior to the introduction of the Luminex HPV Genotyping Assay in national surveillance studies, an in-house evaluation of the assay performance in various residual clinical samples was conducted by PHE. As part of this evaluation, a total of 428 specimens collected as part of the pre-vaccination surveillance were retested using the post-vaccination Luminex-based test to investigate the potential for bias resulting from this assay change. The results of this validation study were published internally within PHE in August 2011.

Importantly, both assays detected a similar positivity rate for the high-risk HPV vaccine types (22.2% for the pre-vaccination assay and 23.8% for the post-vaccination assay) with a high agreement (kappa = 0.81). However, when the other non-vaccine types were assessed, there were some differences in the detection rate. Overall, the post-vaccination assay identified more non-vaccine high-risk HPV positives than the pre-vaccination HC2/Linear array assay (51.6% vs. 45.8%, kappa 0.78). This difference was likely due to the reduced sensitivity of the HC2 assay compared to a direct PCR amplification-based assay. The positivity for all HPV types using the two assays is given in Table C1 in the Appendix.

4.4.3. Adjustment of pre-vaccination prevalence estimates

I have previously described how I proposed to compare the HPV prevalence in the pre-vaccination period to the post-vaccination period using prevalence ratios (Sections 4.3.12 and 4.3.13). In this section, I describe techniques I developed and applied to estimate the prevalence of HPV infection in the pre-vaccination period that we would have expected to observe if we had used the post-vaccination assay. In Section 4.4.4, I describe techniques used to compare the HPV prevalence in the pre- and post-vaccination periods, adjusted for this assay change.

As described in the previous section, the assays used in the pre-vaccination and post-vaccination periods had different diagnostic accuracy for detection of certain

HPV types. Therefore, unadjusted HPV prevalence estimates would not have been comparable between the pre- and post-vaccination period. To account for this, I adjusted pre-vaccination prevalence to estimate the prevalence that would have been observed if the post-vaccination assay had been used. To do this, I use the following formula proposed by Rogan and Gladen[122] to adjust for an imperfect test;

$$p = \frac{(t+\beta-1)}{(\alpha+\beta-1)} \qquad (1)$$

where;

p is the pre-vaccination prevalence if testing using the post-vaccination assay

t is the proportion of specimens testing positive using the pre-vaccination assay

α is the sensitivity of the pre-vaccination assay compared to the post-vaccination assay

β is the specificity of the pre-vaccination assay compared to the post-vaccination assay

This estimate requires a known sensitivity and specificity for the test. In the HPV infection surveillance, the sensitivity and specificity comparing the pre-vaccination and post-vaccination assay were not known but were estimated from a validation study (as described in Section 4.4.2), resulting in the following formula;

$$\widehat{p} = \frac{(\hat{t}+\widehat{\beta}-1)}{(\widehat{\alpha}+\widehat{\beta}-1)} \qquad (2)$$

Rogan and Gladen demonstrated that *(2)* above would be considerably less biased than just using proportion of specimens testing positive using the pre-vaccination assay.

The simplest approach to calculate confidence intervals for this estimate would be to use the asymptomatic variance of $\hat{p}$ obtained by Taylor series expansion;

$$var(\hat{p}) = \frac{\hat{t}(1-\hat{t})/n_t + \hat{p}^2\hat{\alpha}(1-\hat{\alpha})/n_\alpha + (1+\hat{p})^2\hat{\beta}(1-\hat{\beta})/n_\beta}{(\hat{\alpha}+\hat{\beta}-1)^2} \quad (3)$$

$where\ n_t, n_\alpha\ and\ n_\beta\ are\ the\ sample\ sizes\ used\ to\ estimate\ \hat{t}, \hat{\alpha}\ and\ \hat{\beta}\ respectively$

However, Lang and Reiczigel have since demonstrated that the coverage probability of confidence intervals derived from *(3)* could fall below the nominal level in some situations (importantly here, the coverage of these confidence intervals could be incorrect if the samples sizes n_α or n_β are not large)[123]. Consequently, Lang and Reiczigel proposed an approach to estimate confidence intervals with improved coverage when sensitivity and specificity estimates are unknown[123]. I used this approach to calculate the lower and upper confidence limits for prevalence estimated in the HPV infection surveillance. Specifically, if;

$$n_p' = n_p + Z_{crit}^2 \quad (4)$$

$$t' = \frac{(n_p\hat{t}) + Z_{crit}^2/2}{n_p + Z_{crit}^2} \quad (5)$$

Then the confidence interval for t' is;

$$t' \pm Z_{crit} \cdot \sqrt{t' \frac{(1-t')}{n_p'}} \quad (6)$$

Where the sensitivity and specificity are estimated, let;

$$n_\alpha' = n_\alpha + 2 \quad (7)$$

$$n_\beta' = n_\beta + 2 \quad (8)$$

$$\alpha' = \frac{n_\alpha(\alpha+1)}{n_\alpha+2} \quad (9)$$

$$\beta' = \frac{n_\beta(\beta+1)}{n_\beta+2} \quad (10)$$

$$p' = \frac{t'+\beta'-1}{\alpha'+\beta'-1} \quad (11)$$

Substituting the equations *(7)* to *(11)* above to the corresponding terms (i.e. its primed equivalent) in *(3)*, gives the variance of p'. The adjusted confidence intervals for the prevalence will then be;

$$p' + dp \ \pm Z_{crit}.\sqrt{var(p')} \quad (12)$$

where;

$$dp = 2.Z_{crit}^2.\left\{p'.\frac{\alpha'(1-\alpha')}{n'_\alpha} - (1-p').\frac{\beta'(1-\beta')}{n'_\beta}\right\} \quad (13)$$

In this same paper, Lang and Reiczigel show that the mid-point of the confidence interval in *(13)* is more biased than the estimate suggested by Rogan and Gladen above, hence I used *(2)* for prevalence estimates and the approach by Lang and Reiczigel for the confidence intervals. The adjusted prevalence estimates and confidence intervals described above were not included in the papers presented in Section 6.2 or 6.3. However, adjusted prevalence estimates are presented in Table 6.1, alongside the unadjusted prevalence estimates for comparison. Section 4.4.4 below describes further analyses I conducted to adjust for this assay change when comparing HPV infection between the pre- and post-vaccination periods; the resulting odds ratios are included in the paper presented in Section 6.2 and in Table 6.2.

4.4.4. Adjustment of comparison between pre- and post-vaccination prevalence

The techniques described above allowed calculation of prevalence estimates for the pre-vaccination period adjusted for the change in assay. In order to compare HPV infection between the pre- and post-vaccination periods with adjustment for the change in HPV assay, I calculated an odds ratio adjusted for age, collection venue type and chlamydia positivity, using the logitem command in Stata. This command performs logistic regression when the binary outcome is measured with uncertainty by using an expectation-maximisation algorithm to estimate a maximum-likelihood regression model with a known sensitivity and specificity (the sensitivity and specificity for the post-vaccination period were assumed to be 100% with an imperfect sensitivity and specificity for the pre-vaccination period). A similar statistical model to adjust for assay change was not available for use with prevalence ratios (PR), and thus equivalent PR analyses were not carried out.

The estimated ORs provided using the logitem command would not account for the uncertainty surrounding the sensitivity and specificity estimates (estimated from the validation study described in Section 4.4.2). In order to incorporate this additional uncertainty to the standard errors (SEs) of the ORs for each HPV types, I devised a technique using bootstrapping methods, as follows.

(i) I created a validation dataset mimicking the type-specific agreement data from the validation study that compared the two assays

(ii) I selected a bootstrap sample from this validation dataset to calculate a sensitivity and specificity estimate for the pre-vaccination assay compared to the post-vaccination assay [to incorporate the uncertainty of the sensitivity and specificity estimates]

(iii) From my main dataset (i.e. the data including the pre- and post-vaccination test results and individual demographics), I selected a separate bootstrap

sample [to incorporate the uncertainty of the odds ratio due to the sample size of the HPV infection surveillance population]

(iv) I calculated an odds ratio using the logitem command with the sensitivity and specificity estimates from (ii)

(v) I repeated steps (i) to (iv) 1,000 times and extracted the resultant odds ratio from each run

Once the bootstrap was completed, the dataset containing the 1,000 odds ratios was used to calculate confidence intervals (taken as the 2.5^{th} and 97.5^{th} percentile of the ORs). The Stata programme which I wrote to perform this bootstrapping is available in Appendix C2.

In this chapter, I have outlined the HPV infection surveillance methods used to evaluate the National HPV Immunisation Programme in England, which inform research questions 1, 2 and 3 of this thesis. In Chapter 5, I describe work conducted for this PhD to compare HPV vaccination records collected from different sources. The results of this comparison informed interpretation of the vaccine effectiveness analyses and estimation of herd protection effects.

Chapter 5: Validation of HPV vaccination records

In this chapter, I present the methods used to collect individual's HPV vaccination records from Child Health Information Systems (CHIS). I also describe the rationale, methods and results of a study I designed and conducted in order to validate a sample of these vaccination records against records held at general practices. The implications of the results of this study are discussed briefly here and explored in more detail in Chapter 6.

5.1. Background

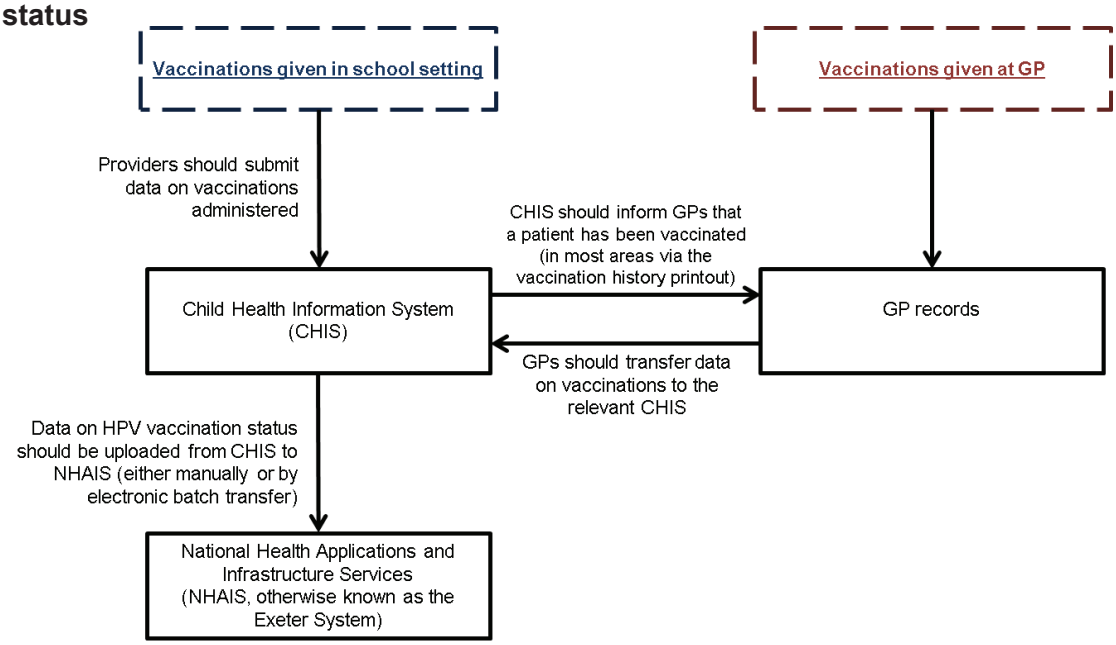
5.1.1. National collection of HPV vaccination status

As previously mentioned in Section 2.7.2, all vaccinations administered to children (defined as those aged 0-18 years) in England should be recorded on a Child Health Information Service (CHIS) System. These systems are held and operated at a local level (within a local authority (LA) or, previously, a primary care trust (PCT)) within Child Health Records Departments (CHRD). The systems do not use standard software and hence there is not a single national CHIS dataset. The vaccination data held on these systems are collated from school nursing services and GP systems. HPV vaccination doses given at schools should also be held on GP records although not all areas will inform GPs of vaccinations performed in school settings.

In addition to ensuring that HPV vaccination status is recorded on CHIS for all vaccinated children, it is the responsibility of CHIS/CHRD to report HPV vaccinations undertaken in schools and other venues to the Cervical Screening System (held on National Health Applications and Infrastructure System, NHAIS, otherwise known as the Exeter system or Open Exeter) so that a woman's vaccination status is available to those conducting cervical screening. As such, complete HPV vaccination records should be held for all vaccinated females on at

least two data systems (i.e. CHIS and Open Exeter), and ideally on GP records as well (Figure 5.1).

Figure 5.1: Process to report and exchange data on individual HPV vaccination status



In practice, the degree of data transfer between systems varies across different areas. In almost all local areas, vaccination was offered in schools for routinely vaccinated girls (i.e. 12-13 year olds) and the younger catch-up cohorts (i.e. ≤16 year olds) and in primary care settings for the older catch-up cohorts (i.e. >16 year olds). I previously contributed towards an unpublished analysis (conducted outside of this PhD) which compared HPV vaccination uptake data from CHIS from eight PCTs with uptake from the Clinical Practice Research Datalink (CPRD) for practices within the same PCTs. The results demonstrated that HPV vaccination uptake using the CPRD data was similar to that in the CHIS data for women in the older catch-up cohorts (i.e. largely vaccinated at general practices) but the uptake in women in the younger cohorts was (i.e. largely vaccinated at schools) was much lower in the CPRD data compared to CHIS data (Table 5.1).

Table 5.1: HPV vaccination uptake among the routine, younger catch-up and older catch-up cohorts for Child Health Information System (CHIS) and Clinical Practice Research Datalink (CPRD)

HPV vaccination cohort	CHIS % (95%CI)	CPRD % (95%CI)
Routine (12-13 years)	88.4 (88.1-88.7)	60.6 (60.1, 61.2)
Younger catch-up (14-16 years)	88.4 (88.0-88.8)	50.4 (49.8, 51.0)
Older catch-up (≥16 years)	55.8 (55.1-56.4)	52.0 (51.5-52.5)

Importantly, the results of this unpublished analysis also demonstrated that recorded uptake of HPV vaccination in some general practices appeared to be very low. Table 5.2 shows categories of HPV vaccination uptake (<20%; 20-40%; 40-60%; 60-80% and 80-100%) for practices in the eight PCTs included in the CPRD analysis.

Table 5.2: Categories of HPV vaccination uptake at general practices in eight PCTs using Clinical Practice Research Datalink (CPRD) data, by vaccination cohort

HPV vaccination uptake (%)	Routine (12-13 yrs)	Younger catch-up (14-16 yrs)	Older catch-up (≥16 yrs)
	Number of practices (%)	Number of practices (%)	Number of practices (%)
<20%	77 (18.5%)	125 (30.0%)	66 (15.5%)
20-40%	37 (8.9%)	33 (7.9%)	54 (12.7%)
40-60%	35 (8.4%)	41 (9.8%)	115 (27.1%)
60-80%	135 (32.5%)	135 (32.4%)	148 (34.8%)
80-100%	132 (31.7%)	83 (19.9%)	42 (9.9%)

Whilst it was expected that some areas/practices would have lower uptake than others, there was an unexpectedly high proportion of practices with <20% uptake (18.5% of practices for routine cohorts, 30.0% for younger catch-up cohorts, and 15.5% for older catch-up cohorts). This suggests that the level of data transfer between CHIS and GP records was inadequate at some practices; particularly for younger vaccination cohorts (<16 years old) for which vaccination uptake was expected to be higher. This could be that data were not sent from CHIS to GPs or that data were sent in paper format and not transferred to electronic GP records. Further to data in Table 5.1 and Table 5.2, conversations with GPs, CHRDs and other local providers, have also provided some insight into the process of recording and transferring HPV vaccination records. These conversations suggested that vaccination records for routine cohorts and younger catch-up cohorts (i.e. those largely vaccinated at schools) are more complete on CHIS but may not always be available on GP systems (this supports results in Table 5.1 and Table 5.2). Furthermore, these conversations suggested that vaccination records for older catch-up cohorts (i.e. those vaccinated at general practices) are usually available on GP systems but in some local areas these data are not always uploaded to CHIS. This is in contrast to the results from the eight PCTs presented in Table 5.1 and suggests that these results may not be generalisable to all practices. In addition to this, the data which are uploaded from CHIS to Open Exeter appears incomplete in almost all local areas, particularly for the catch-up cohorts[78]. This varies by local area and birth cohort but the data from the 2013/14 vaccination cohort showed that HPV vaccination coverage on Open Exeter was between 4.2% and 86.5% lower by area team than the aggregated coverage data reported via ImmForm (Section 2.7.2)[78].

For the HPV infection surveillance described in Chapter 4, I made use of HPV vaccination data held in CHIS. I described this process briefly in Section 4.3.6, and

in more detail below in Section 5.2.2. I also performed a validation study to compare HPV vaccination records in CHIS with GP records for samples collected from one of the laboratories included in this surveillance (this validation study is described in Section 5.3).

5.1.2. Why is accurate recording of HPV vaccination records required?

As women reach the age at which they are invited for cervical screening, access to an individual's vaccination records becomes important for two reasons. Firstly, PHE are conducting enhanced surveillance to perform HPV testing on all cervical cancers diagnosed in women who would have been eligible to have received the HPV vaccine. This surveillance does not fall within the scope of this PhD but accurate individual vaccination records are essential to identify potential vaccine failures (i.e. routinely vaccinated women who are diagnosed with an HPV16/18 positive cervical cancer). Secondly, although currently cervical screening protocols are identical for vaccinated and unvaccinated women, there is a possibility that screening procedures may differ depending on vaccination status in the future (particularly if the nonavalent vaccine were introduced in the UK)[124]. For this to be possible, accurate HPV vaccination records would need to be linked to a woman's cervical screening records (i.e. Open Exeter).

Furthermore, I have previously described in Section 4.2.1 the benefits of collecting HPV vaccination status for national HPV infection surveillance. Inaccurate recording of HPV vaccination status could affect the calculation of vaccine effectiveness, particularly if there was a bias in reporting (e.g. if those at higher risk of HPV infection were more likely to have inaccurate HPV vaccination status). This latter point is explored further in Section 6.4.4.

5.2. (Methods 1) Collection of HPV vaccination records from Child Health Information Systems for HPV infection surveillance

5.2.1. Eligible population

I previously described methods for the HPV infection surveillance conducted at 10 laboratories across England and, briefly, the linkage with individual HPV vaccination records in CHIS (Section 4.3.6). In this section I provide more detail on the methods to collect HPV vaccination records for individuals included in this surveillance who met the following criteria:

- *Valid NHS number*

  NHS number was the only patient identifier which could uniquely identify records in the CHIS and the HPV surveillance study and hence was required for linkage. I assessed that available NHS numbers were 10 digit numbers with an eligible 10th check digit[125].

- *Known local authority of residence (formerly Primary Care Trust)*

  As described, the majority of local areas held records in separate CHIS databases; therefore in these areas, a known LA/PCT of residence was required to be able to contact the holder of the relevant CHIS data.

- *Eligible to receive the HPV vaccine as part of the National HPV Immunisation Programme*

  This included all women born on or after the 1st September 1990.

Of all eligible residual VVS specimens collected for the HPV infection surveillance (see Section 4.3) that were taken from women who would have been offered the HPV vaccination as part of the national programme, 39% (4,457/11,541) were from women from whom vaccination records could potentially be obtained (i.e. had a valid NHS number). The remaining 61% (7,084/11,541) of specimens were released

for HPV testing with an unknown vaccination status (Section 4.3.7). Of those with a valid NHS number, vaccination records were only requested for 77% (3,432/4,457) of women as for remaining women, because either the local authority of residence was unknown or the local CHRD did not agree (or were unable) to link with the local CHIS system. The full details of linkage to vaccination records, stratified by laboratory are provided in Table 5.3.

Table 5.3: Collection of HPV vaccination records by testing laboratory and local authority (including data to end 2016)

Laboratory Name	Local authority (LA) or Primary Care Trust (PCT)	Number of eligible samples received	Number (%) born on or after 1st September 1990	Number (%) eligible and with valid NHS number	CHIS contact approached (N/Y)?	CHIS contact agreed to participate (N/Y)?	Number (%) returned
Addenbrookes	Cambridge	313	164 (52%)	5 (3%)			
	Huntingdonshire	292	169 (58%)	*masked*[4]			
	Fenland	171	98 (57%)	0 (0%)			
	South Cambridgeshire	126	64 (51%)	0 (0%)	Y[3]	Y[3]	130 (49%)[3]
	East Cambridgeshire	84	43 (51%)	*masked*[4]			
	Other[1]	23	15 (65%)	0 (0%)			
	Unknown†	353	329 (93%)	259 (79%)			
Aintree	Liverpool	409	268 (66%)	44 (16%)	Y	N	
	Cheshire West and Chester	315	203 (64%)	40 (20%)	Y	N	
	Wirral	203	127 (63%)	12 (9%)	Y	Y	10 (83%)
	Cheshire East	149	96 (64%)	18 (19%)	Y	N	
	Sefton	124	80 (65%)	0 (0%)	N		
	St. Helens	40	27 (68%)	*masked*[4]	N		
	Halton	31	18 (58%)	0 (0%)	N		
	Knowsley	36	18 (50%)	0 (0%)	N		
	Warrington	22	17 (77%)	0 (0%)	N		
	Other[1]	61	43 (70%)	*masked*[4]	N		
	Unknown	57	32 (56%)	16 (50%)	-		
Cornwall	Cornwall	3,454	2501 (72%)	2267 (91%)	Y	Y	1932 (85%)
	Other[1]	54	36 (67%)	32 (89%)	N		
	Unknown	31	27 (87%)	16 (59%)	-		

East Kent	Canterbury	1144	929 (81%)	289 (31%)	Y	N	
	Thanet	616	521 (85%)	41 (8%)	Y	N	
	Ashford	485	382 (79%)	19 (5%)	Y	N	
	Dover	438	346 (79%)	18 (5%)	Y	N	
	Shepway	396	299 (76%)	28 (9%)	Y	N	
	Swale	279	253 (91%)	49 (19%)	Y	N	
	Other[1]	71	65 (92%)	6 (9%)	N		
	Unknown	461	400 (87%)	96 (24%)	-		
Leeds	Leeds	2345	1783 (76%)	184 (10%)	Y	N	
	Bradford	260	179 (69%)	8 (4%)	N		
	Other[1]	120	86 (72%)	0 (0%)	N		
	Unknown	271	225 (83%)	*masked*[4]	-		
Lewisham	Lewisham	382	146 (38%)	NA[2]	NA[2]	NA[2]	82 (56%)
	Greenwich	39	18 (46%)	NA[2]	NA[2]	NA[2]	10 (56%)
	Bromley	36	16 (44%)	NA[2]	NA[2]	NA[2]	8 (50%)
	Southwark	29	13 (45%)	NA[2]	NA[2]	NA[2]	5 (38%)
	Other[1]	31	10 (32%)	NA[2]	NA[2]	NA[2]	6 (60%)
	Unknown	201	124 (62%)	NA[2]	NA[2]	NA[2]	66 (53%)
Norfolk and Norwich	Waveney	124	87 (70%)	0 (0%)	N		
	Great Yarmouth	92	65 (71%)	0 (0%)	N		
	Norwich	46	33 (72%)	0 (0%)	N		
	South Norfolk	23	12 (52%)	0 (0%)	N		
	Broadland	20	16 (80%)	0 (0%)	N		
	Other[1]	43	30 (70%)	0 (0%)	N		
	Unknown	*masked*[4]	*masked*[4]	0 (0%)	N		
Portsmouth	Portsmouth	107	61 (57%)	59 (97%)	Y	Y	17 (29%)

	Havant	54	32 (59%)	31 (97%)	Y	N	
	Gosport	52	33 (63%)	31 (94%)	Y	N	
	Fareham	36	18 (50%)	17 (94%)	Y	N	
	Other[1]	17	11 (65%)	11 (100%)	N		
	Unknown	0	NA	NA	-		
Stoke	Stoke-on-trent	496	319 (64%)	214 (67%)	Y	Y	54 (25%)
	Newcastle-under-Lyme	236	172 (73%)	135 (78%)	Y	Y	21 (16%)
	Staffordshire Moorlands	119	101 (85%)	85 (84%)	Y	Y	7 (8%)
	Stafford	86	78 (91%)	65 (83%)	Y	Y	0 (0%)
	Other[1]	68	50 (74%)	24 (48%)	N		
	Unknown	8	*masked*[4]	*masked*[4]	-		
UCL	Islington	137	96 (70%)	0 (0%)	N		
	Camden	135	44 (33%)	0 (0%)	N		
	Hackney	45	27 (60%)	0 (0%)	N		
	Haringey	42	24 (57%)	0 (0%)	N		
	Other[1]	115	52 (45%)	0 (0%)	N		
	Unknown	10	5 (50%)	0 (0%)	N		

NA = Not applicable

1: Includes LAs/PCTs with <10 women with potential vaccination records

2: Lewisham laboratory had access to patient's HPV vaccination records. Therefore, vaccination records were sent directly on data collection forms and NHS number was not required for linkage

3: CHIS data from Addenbrookes were searched across all local areas

4: In accordance with PHE data sharing policy, cells with values between 1 and 4 inclusive were masked. If masked cells could be deduced from values of other cells then the next smallest cell was also masked

5.2.2. Methods for HPV vaccination data collection from CHIS and linkage with the HPV infection surveillance database

For each area (either LA or PCT) with eligible specimens to link to HPV vaccination records, it was necessary to contact the person responsible for the relevant Child Health Information System. If it was not clear who the relevant contact was, I contacted the Screening and Immunisation Teams (SITs). SITs are employed by PHE and provide local leadership for screening and immunisation services within an Area Team (AT). Therefore, SITs have close relationships with screening and immunisation colleagues within their area and often had the ability to put me in touch with the relevant CHIS contact.

Once the relevant contact at CHIS was identified, I contacted them (either by telephone or initially by email and then following up on the telephone if necessary) to describe the rationale, methods and legal/ethical basis of the HPV infection surveillance. If the CHIS department was able to assist with the data linkage then initially I liaised with PHE Business Development colleagues to produce data sharing agreements for that area. Only when this was in place did I request any data linkage or transfer. As data extraction from CHIS required a significant amount of work for NHS and/or PHE SIT colleagues, I did not contact local authorities where there were only a small number of eligible patients (i.e. less than 10).

Once the above was in place, I sent a list of valid NHS numbers for the eligible women included in the surveillance to the relevant CHIS contact by secure encrypted email, requesting the following information:

- Vaccination status (unvaccinated, partially vaccinated, fully vaccinated)
- Date received (dose 1)
- Batch number (dose 1) (if known)
- Date received (dose 2)
- Batch number (dose 2) (if known)

- Date received (dose 3)
- Batch number (dose 3) (if known)
- Vaccine type (bivalent or quadrivalent)
- GP name (for Cornwall local authority only – see Section 5.3)
- GP practice name (for Cornwall local authority only – see Section 5.3)

On receipt of data from CHIS, I linked the returned data with the HPV surveillance dataset using the NHS number. Once the vaccination data were linked, patient identifiable information (including date of birth, NCSP/CTAD test identifier and NHS number) were irreversibly deleted (with the exception of those included in the validation study; Section 5.3). Only once the patient identifiable data were deleted were these specimens released for testing by the VRD laboratory (Section 4.3.8).

5.2.3. Results of HPV vaccination coverage from CHIS for the national HPV infection surveillance

Across all laboratories, 68% (2,348/3,432) of HPV vaccination records which were requested were returned and linked. I discuss the representativeness of these records in Section 6.3 and Section 6.4.4. Full details of the number of records requested and returned, stratified by laboratory, are shown in Table 5.3. Of those returned, the proportion of women who had received at least one dose of the vaccine was 82% (1,924/2,348) although this varied by the age at which vaccination was offered (93% in routine cohorts, 89% in younger catch-up cohorts and 69% in older catch-up cohorts). Vaccination coverage stratified by age-group and laboratory is shown in Table 5.4.

Further details of the characteristics of vaccinated and unvaccinated women, and of women with and without recorded vaccination status are given in Section 6.3 and Section 6.4.4. I also present the results of HPV prevalence in vaccinated women compared to unvaccinated women in Section 6.3.

Table 5.4: HPV vaccination coverage in CHIS for individuals included in HPV infection surveillance, stratified by age at vaccination and testing laboratory[1]

	Unvaccinated	Vaccinated (at least one dose)
Lewisham[2]		
12 year-olds	*masked*[3]	*masked*[3]
14-15 year olds	*masked*[3]	*masked*[3]
16-17 year olds	81 (67.5%)	39 (32.5%)
All ages	*96 (60.4%)*	*63 (39.6%)*
Cambridge		
12 year-olds	6 (4.6%)	124 (95.4%)
14-15 year olds	0	0
16-17 year olds	0	0
All ages	6 (4.6%)	124 (95.4%)
Aintree		
12 year-olds	0	0
14-15 year olds	0	*masked*[3]
16-17 year olds	5	*masked*[3]
All ages	5 (50.0%)	5 (50.0%)
Cornwall		
12 year-olds	51 (7.8%)	603 (92.2%)
14-15 year olds	45 (9.1%)	448 (90.9%)
16-17 year olds	184 (23.4%)	601 (76.6%)
All ages	280 (14.5%)	1,652 (85.5%)
Portsmouth		
12 year-olds	0	0
14-15 year olds	*masked*[3]	16
16-17 year olds	0	0
All ages	*masked*[3]	16
Stoke		
12 year-olds	0	*masked*[3]
14-15 year olds	*masked*[3]	*masked*[3]
16-17 year olds	*masked*[3]	*37*
All ages	*22 (26.8%)*	*60 (73.2%)*

1: Restricted to laboratories for which vaccination status was available

2: In Lewisham, data were collected directly from laboratory using data on chlamydia-test request form

3: In accordance with PHE data sharing policy, cells with values between 1 and 4 inclusive were masked. If masked cells could be deduced from values of other cells then the next smallest cell was also masked

5.3. (Methods 2) Validation of HPV vaccination records using GP records

5.3.1. Rationale

As previously described, there was some concern that vaccination records in CHIS may not be accurate in some areas, particularly for women vaccinated outside of schools (see Section 5.1.1). Given the importance of accurate HPV vaccination data for HPV infection surveillance, I designed and conducted a validation study to ascertain the accuracy of HPV vaccination records on CHIS compared to data collected at general practices in Cornwall.

5.3.2. Setting for the validation study

As there is no national general practice database, it was not possible to check all CHIS records against GP records. Therefore I decided to select a sample of women for whom vaccination status in the different systems could be compared. This sample of women had to be from a local authority with available CHIS data for linkage with the HPV infection surveillance data (Section 5.2.1). Cornwall local authority had the highest proportion of CHIS records linked to the HPV infection surveillance data. In addition, the administration of HPV vaccination in the Cornwall local authority (formerly Cornwall PCT) was unique in that it was a GP-based programme for all ages (i.e. HPV vaccination was not performed in schools as it was in other local authorities). Therefore, I decided to conduct a validation study to ascertain whether HPV vaccination records held at general practices were accurately recorded on CHIS in Cornwall.

I contacted the SIT who had facilitated collection of CHIS data for the Cornwall local authority to discuss validation of the CHIS data using GP records in this area. The validation was supported by the Screening and Immunisation Lead (SIL) of the SIT and, on the advice of the SIL, the SIL and I jointly approached the Cornwall Local Medical Committee (LMC) and the South West Regional Medical Director for NHS

England. There were some concerns expressed about the burden for GPs if they were asked to provide detailed HPV vaccination records for each patient (including dates for each dose received). Therefore, in response I proposed (i) to limit the number of GPs included and the number of records searched at each practice and, (ii) to ask GPs to provide only vaccination status (unvaccinated, 1-dose received, 2-doses received, 3-doses received) rather than full details of the dates and batch numbers for each dose given. With this change, the Cornwall LMC and NHS England South (SW) Medical Director both supported this study. The original permissions for this HPV infection surveillance (Section 4.3.2) included collection of GP details to enable collection of HPV vaccination records. Therefore, once it was agreed that this study could proceed, I requested data (in addition to the data collected in Section 5.2.2) on patients' GPs (including the GP name and practice name) from the Cornwall CHIS.

5.3.3. Sample size for the validation study

Table 5.5 gives the number of CHIS records held for Cornwall local authority in April 2016 (in the column "Total Population"). Patients were from 67 general practices across Cornwall local authority (with between 1 and 133 eligible patients from each practice). As described in the previous section, to perform a validation of all CHIS records at all general practices was considered impractical given the additional work required by GPs. Selecting approximately 40% of specimens allowed sufficient precision around estimates of the expected agreement (Table 5.5). However, to achieve this sample size would have either involved collection of 40% of records from all 231 practices, or, all records from 40% of practices (which would be up to 133 records from the largest practice). Both of these options were considered impractical, hence ~40% of partially vaccinated females (n=37) and unvaccinated females (n=95) were selected but only ~10% of fully vaccinated females (n=127) which provided sufficient power for the required precision in all groups.

Table 5.5: Estimated precision comparing HPV vaccination status on CHIS vs. GP, for given sample sizes

	Total population		Validation population			
					Precision for given sample size	
Status	CHIS (%)	Number of specimens	Estimated Agreement (%)	Sample size	95% CI	95% CI with finite population correction
Fully vaccinated	79.8%	1249	90	127	84.8-95.2	85.1-95.0
Fully vaccinated	79.8%	1249	80	127	73.1-87.0	73.4-86.6
Fully vaccinated	79.8%	1249	70	127	62.0-78.0	62.4-77.6
Fully vaccinated	79.8%	1249	60	127	51.5-68.5	51.9-68.1
Partially vaccinated	5.9%	92	90	37	80.3-99.7	81.1-98.9
Partially vaccinated	5.9%	92	80	37	67.1-92.9	68.2-91.8
Partially vaccinated	5.9%	92	70	37	55.2-84.8	56.5-83.5
Partially vaccinated	5.9%	92	60	37	44.2-75.8	45.5-74.5
Unvaccinated	14.4%	225	90	95	84.0-96.0	85.4-94.6
Unvaccinated	14.4%	225	80	95	72.0-88.0	73.9-86.1
Unvaccinated	14.4%	225	70	95	60.8-79.2	63.0-77.0
Unvaccinated	14.4%	225	60	95	50.2-69.9	52.5-67.5
			Total	259		

5.3.4. Methods for HPV vaccination data collection from GPs

For ease of data collection, practices were sampled rather than individual patients. The patient records to include in the validation study were selected as follows:

(i) Practices were categorised according to the number of registered patients with HPV vaccination records in CHIS (≤7 patients; 8-17 patients; 18-30 patients; >30 patients)

(ii) I randomly selected 6 practices within each group defined in (i) (i.e. 24 practices in total). This included 18% of practices (i.e. 24 of 133 practices) but by selecting proportionately more of the larger clinics, this included around 40% of women.

(iii) From each selected practice, I selected all unvaccinated patients, all partially vaccinated patients and a random selection of approximately 25% of fully vaccinated patients (i.e. representing 40% of partially and unvaccinated women and about 10% of fully vaccinated women from all practices).

I drafted a letter for GPs, describing the remit of PHE to monitor the HPV Immunisation Programme and the importance of this HPV infection surveillance. The content of this letter was approved by the Screening and Immunisation Lead (SIL) and was signed by me as well as the SIL (Appendix D1). I also drafted a data collection form which included patients' NHS number and a choice of boxes to tick to indicate patients' vaccination status (no record of patient; unvaccinated; one-dose received; two-doses received; three-doses received) (Appendix D1). I then sent this letter and the data collection form to each practice. The letter and form were addressed to a named person within the practice and double enveloped (both the internal and external envelope were addressed to the same named person but only the internal envelope was marked "Private and Confidential").

I sent letters to the 24 selected practices to request HPV vaccination status for 259 women. Full details are given in Table 5.6. GPs were sent two return envelopes which were addressed to me at PHE (so the form could be returned double enveloped as above). Data were entered by colleagues at PHE onto a specifically designed spreadsheet. If forms were not returned within two weeks, I contacted the GP by telephone or secure email (nhs.net to nhs.net) to check the status of the response. I continued to follow up directly with the GP until data collection forms were returned.

Table 5.6: Requests sent to GPs in Cornwall for selected patients' HPV vaccination status

General Practice	Number of patients requested			
	Total	*Fully vaccinated*	*Partially vaccinated*	*Unvaccinated*
Practice 1	51	*25*	*11*	*15*
Practice 2	33	*22*	*3*	*8*
Practice 3	28	*10*	*3*	*15*
Practice 4	17	*8*	*3*	*6*
Practice 5	16	*5*	*5*	*6*
Practice 6	14	*3*	*4*	*7*
Practice 7	14	*8*	*1*	*5*
Practice 8	14	*10*	*1*	*3*
Practice 9	12	*6*	*2*	*4*
Practice 10	10	*2*	*2*	*6*
Practice 11	9	*6*	*0*	*3*
Practice 12	7	*3*	*0*	*4*
Practice 13	7	*4*	*0*	*3*
Practice 14	6	*1*	*1*	*4*
Practice 15	5	*3*	*0*	*2*
Practice 16	4	*1*	*0*	*3*
Practice 17	4	*4*	*0*	*0*
Practice 18	2	*1*	*1*	*0*
Practice 19	1	*0*	*0*	*1*
Practice 20	1	*1*	*0*	*0*
Practice 21	1	*1*	*0*	*0*
Practice 22	1	*1*	*0*	*0*
Practice 23	1	*1*	*0*	*0*
Practice 24	1	*1*	*0*	*0*
Total	259	127	37	95

5.4. Results

5.4.1. Response rate

Either in direct response to the initial letter or after follow-up communication, I received data from all 24 practices with HPV vaccination status for 223/259 (85%) of patients (35 patients were no longer registered at the practice and vaccination records were not available, 1 patient's vaccination status was unknown).

5.4.2. Results of validation study comparing CHIS and GP records of HPV vaccination status

The vaccination status on CHIS compared to GP systems is provided in Table 5.7. Women who were recorded as unvaccinated on CHIS were slightly more likely to have a missing record on their corresponding GP system (i.e. were no longer registered at the practice or vaccination records were not available; 18.9% of unvaccinated women had a missing record compared to 11.8% for fully vaccinated women and 8.1% for partially vaccinated women). I discuss the potential bias of these missing records in Section 5.4.3 below. Among those with GP records available, there was generally very good agreement for women recorded as fully vaccinated in CHIS (107/112 (95.5%) were also recorded as fully vaccinated on GP records) and those recorded as unvaccinated (66/77 (85.7%)). However, of 34 women recorded as partially vaccinated in CHIS who had a GP record, 9 (26.5%) were recorded as fully vaccinated and 4 (11.8%) were recorded as unvaccinated, with only 61.8% agreement for partial vaccination status.

Table 5.7: HPV vaccination status on CHIS compared to GP systems (n=259)

CHIS vaccination Status	No record at practice (n=36)[1]	n (%)	Vaccination status at general practice		
			Unvaccinated (n=74)	Partially vaccinated (n=27)	Fully vaccinated (n=122)
Unvaccinated (n=95)	18 (50.0%)	77 (34.5%)	n=66 85.7% (75.9-92.6)	n=5 6.5% (2.1-14.5)	n=6 7.8% (2.9-16.2)
Partially vaccinated (n=37)	3 (8.3%)	34 (15.2%)	n=4 11.8% (3.3-27.5)	n=21 61.8% (43.6-77.8)	n=9 26.5% (12.9-44.4)
Fully vaccinated (n=127)	15 (41.7%)	112 (50.2%)	n=4 3.6% (1.0-8.9)	n=1 0.9% (0.0-4.9)	n=107 95.5% (89.9-98.5)

1: *35 patients were no longer registered at the practice and vaccination records were not available, 1 patient's vaccination status was unknown*

5.4.3. Discussion

As stated in the background of this Chapter, collection of accurate HPV vaccination records is important for national HPV infection surveillance to enable accurate monitoring of the population-level effects of HPV vaccination and calculation of the vaccine effectiveness against HPV infection. Nationally, it is not clear whether CHIS or GPs are the “gold standard” data source for HPV vaccination records; as described in Section 5.1.1, this is likely to vary according to area and HPV vaccination cohort (due to the venue of vaccine delivery). CHIS seems to be, on average, more accurate for school-based vaccination but in some areas, GP records are likely to be more accurate for older catch-up cohorts. CHIS has the advantage of having a single database for all individuals within a local authority. However, given the known inadequate reporting of vaccination receipt between different systems, it is difficult to assume that an absence of an HPV vaccination record in a system implies that a woman is unvaccinated without checking other data sources.

The validation study described in this chapter aimed to assess the robustness of calculating effectiveness of HPV vaccination against HPV infection using vaccination status data from CHIS (as described in Section 4.3.15), specifically in Cornwall where vaccinations were offered in general practice. There was strong agreement between the CHIS and GP records for those recorded as fully vaccinated and those recorded as unvaccinated. Given that vaccination was offered via general practice, it is surprising that there were any women recorded as unvaccinated at the practice but partially or fully vaccinated on CHIS, although there were relatively few of these (n=8/74). Furthermore, although the numbers of women recorded as partially vaccinated were also relatively small, those recorded as partially vaccinated on CHIS were often recorded as fully vaccinated on GP systems, likely suggesting some missed doses on CHIS. I discuss below some

limitations of this validation study. I discuss the potential implications of misclassification of HPV vaccination status, based on results from this validation study, in Section 6.4.4.

This validation study had some limitations. Firstly, this study was conducted in one local authority only; the only local authority in the country that offered routine HPV vaccination via general practice for women of all ages (rather than in school settings for younger women). Therefore, these results may not be generalisable to younger women from other local authorities included in the national HPV infection surveillance where vaccinations for younger cohorts would have been offered in schools. The results may be more likely to inform data collection for older catch-up cohorts vaccinated outside of schools although, as described in Section 5.1.1, the transfer of data is variable across different areas. Secondly, although there was 100% response from GPs, there were still some missing data for individuals (36 out of 259 women (13.9%)), mostly due to women no longer being registered at the same GP as when they were vaccinated, a particular issue when collecting vaccination status retrospectively. The proportion of women recorded as unvaccinated on CHIS was somewhat higher among those with missing GP data compared to those with non-missing GP data (50.0% vs. 34.5% respectively; p-value 0.074). The vast majority of missing GP data was because women were no longer registered at the general practice. If moving practice was unrelated to whether GP and CHIS records were discrepant (i.e. non-differential misclassification) then the missing data would not affect the agreement estimates in Table 5.7, other than to widen the confidence interval. However, if women with missing GP records were more likely to be unvaccinated (for example, if they were not offered HPV vaccination because they moved practice; i.e. differential misclassification) then this could have underestimated the agreement for unvaccinated women and overestimated the agreement for vaccinated women (both

partially and fully vaccinated, given that 18 of the 36 women with no GP records were recorded as vaccinated on CHIS). Thirdly, despite sampling a higher proportion of unvaccinated women and partially vaccinated women compared to fully vaccinated women, the numbers in these groups were still relatively small. Therefore, there is more uncertainty around the agreement for these women, as expected from precision estimates conducted prior to conducting this study (Table 5.5). Finally, in order to limit the additional work for GPs, we only collected vaccination status rather than full details so it was not possible to validate details of age at vaccination and time between doses (which would have also required an even larger sample size).

CHIS data have clear advantages when collecting vaccination data for large populations of women. In this study, I only had to approach one CHIS to obtain HPV vaccination status for all women living in the Cornwall local authority but I would have had to contact 67 GPs to obtain all the corresponding data from GP records. However, the difficulty in obtaining CHIS records described in Section 5.1.1, particularly collecting data retrospectively, highlights the importance of ensuring these data are transferred to Open Exeter and available when women attend for cervical screening (this is discussed further in Section 9.4).

In this chapter, I have provided further description of the methods I adopted to collect HPV vaccination status, an important aspect of the HPV infection surveillance to allow estimation of the direct effect of HPV vaccination and indirect effect of herd protection. I have also presented the results of a study which compared HPV vaccination status from different sources. In the following chapter (Chapter 6), I present these results which address research questions 1, 2 and 3 of this thesis. In the discussion of this next chapter (Section 6.4.4), I explore how the results of the validation study presented above could have affected the interpretation of the results of the HPV infection surveillance.

Chapter 6: Results of HPV infection surveillance to evaluate the National HPV Immunisation Programme

6.1. Introduction

In this chapter, I present the results of the ongoing repeat cross-sectional surveillance of type-specific HPV infections among young sexually active women in England. This chapter includes two papers.

In the first paper, published in BMJ Open in 2016, I extended analyses I had originally conducted prior to the start of this PhD. The original analyses were based on the first 4,178 post-vaccination specimens from sexually active young females in England (2010-2011), and focussed largely on changes in HPV16/18 prevalence (with limited power to consider changes in other HPV types)[126]. In the new analysis conducted for the first paper in this Chapter, I included additional post-vaccination samples (a total of 7,321 post-vaccination specimens collected up to April 2013) and explored changes in non-vaccine types. I also performed an adjusted analysis to account for the changes in the HPV assay between the pre- and post-vaccination periods (using the methods outlined in Section 4.4). This paper focussed on changes in prevalence between the pre-vaccination period and the post-vaccination period for twenty HPV types. The supplementary Table for this publication, which outlines changes in non-vaccine HPV types among women with at least one HPV type detected, is included in Appendix E.

In the second paper in this chapter (*submitted for publication*), I further updated this analysis, including 15,549 post-vaccination specimens taken up to December 2016, and also included direct calculation of vaccine effectiveness. There were three main elements of this analysis. Firstly, for all women, I focussed on the changes in type-specific HPV prevalence within the post-vaccination period and the association between these changes and population vaccination coverage. The HPV testing

assay has remained constant within the post-vaccination period so unlike the first paper, there was no need to adjust for the change in HPV assay between time periods. Secondly, for a subset of women with known vaccination status, I calculated vaccine effectiveness. Finally, I also assessed the herd protection effect in unvaccinated women.

At the end of the chapter, I include some further analyses and discussion around three areas; (i) Changes over time which could affect the results in the above two papers (including the change in the HPV assay between the pre- and the post-vaccination period, changes in chlamydia positivity over time, and potential confounding due to changes in other unrecorded patient demographics and behaviour); (ii) the potential misclassification of HPV vaccination status from CHIS was described in detail in Chapter 5; and (iii) further interpretation of the changes in non-vaccine types, including exploration of possible methods that could be used to quantify the potential effect of type-replacement and/or unmasking in the post-vaccination period.

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SECTION A – Student Details

Student	David Mesher
Principal Supervisor	Sara Thomas
Thesis Title	Assessment of the population-level impact of a high coverage HPV immunisation programme in young females

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

Where was the work published?	BMJ Open		
When was the work published?	February 2016		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	N/A		
Have you retained the copyright for the work?*	**Yes**	Was the work subject to academic peer review?	**Yes**

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SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	
Please list the paper's authors in the intended authorship order:	
Stage of publication	Choose an item.

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	The post-vaccination surveillance (commencing in October 2010) was initiated and designed by Kate Soldan. Since October 2011, I was responsible for liaising with local laboratories providing samples for the post-vaccination surveillance. I was also responsible for all data collection and management. I performed the data linkage

	with the Chlamydia Testing Activity Dataset (CTAD). I performed anonymisation of patient identifiable data and liaised with the PHE laboratory to inform them when samples had been anonymised and could be tested. Kavita Panwar and Simon Beddows performed the laboratory testing. I conducted all data management and statistical analyses with advice from Sara Thomas and Kate Soldan. I wrote the first draft of the manuscript which was commented on by all authors. This paper was peer reviewed and I incorporated suggestions from reviewers and responded to their comments, with input from other authors.

Student Signature: [redacted] **Date:** 12/04/2018

Supervisor Signature: [redacted] **Date:** 12/4/18

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BMJ Open

Continuing reductions in HPV 16/18 in a population with high coverage of bivalent HPV vaccination in England: an ongoing cross-sectional study

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ABSTRACT

Objectives: The human papillomavirus (HPV) immunisation programme in England was introduced in 2008. Monitoring changes in type-specific HPV prevalence allows assessment of the population impact of this vaccination programme.

Methods: Residual vulva-vaginal swab specimens were collected from young sexually active women (aged 16–24 years) attending for chlamydia screening across England. Specimens were collected between 2010 and 2013 for type-specific HPV-DNA testing. HPV prevalence was compared to a similar survey conducted in 2008 prior to the introduction of HPV vaccination.

Results: A total of 7321 specimens collected in the postvaccination period, and 2354 specimens from the prevaccination period were included in this analysis. Among the individuals aged 16–18 years, with an estimated vaccination coverage of 67%, the prevalence of HPV16/18 infection decreased from 17.6% in 2008 to 6.1% in the postvaccination period. Within the postvaccination period, there was a trend towards lower HPV16/18 prevalence with higher vaccination coverage and increasing time since vaccine introduction from 8.5% in the period 2–3 years postvaccination to 4.0% in the period 4–5 years postvaccination. The prevalence of HPV31 reduced from 3.7% in the prevaccination period to 0.9% after vaccine introduction, although this no longer reached statistical significance after additional consideration of the uncertainty due to the assay change. Smaller reductions were seen in the individuals aged 19–21 years with lower estimated vaccination coverage, but there was no evidence of a reduction in the older unvaccinated women. Some overall increase in non-vaccine types was seen in the youngest age groups (ORs (95% CI); 1.3 (1.0 to 1.7) and 1.5 (1.1 to 2.0) for individuals aged 16–18 and 19–21 years, respectively, when adjusted for known population changes and the change in assay) although this should be interpreted with caution given the potential unmasking effect.

Conclusions: These data demonstrate a reduction in the HPV vaccine types in the age group with the highest HPV vaccination coverage.

Strengths and limitations of this study

- We conducted human papillomavirus (HPV) surveillance among a large number of young women attending for chlamydia screening, with HPV type-specific testing performed for almost 10,000 women.
- The large sample size of this study has allowed us to consider the population impact of the bivalent HPV vaccine against the two vaccine types, and against cross-protective HPV types.
- We demonstrate continued decreases in the prevalence of vaccine-targeted HPV types over time up to 4 years after the introduction of the bivalent vaccination programme.
- Analyses compare data from repeat cross-sectional surveys. Therefore, unrecorded changes in the population characteristics may have resulted in a change in HPV prevalence which is unrelated to HPV vaccination.

INTRODUCTION

Persistent infection with a high-risk (HR) human papillomavirus (HPV) type is a necessary cause of cervical cancer, and has been shown to be associated with other cancers in men and women.[1 2] Two of these HR-HPV types, HPV16 and HPV18, are present in around 70–80% of cervical cancers.[3 4] Infection with low-risk (LR) HPV6 or HPV11 has been shown to be associated with the vast majority of genital warts.[5]

HPV vaccination of young females has been introduced widely in developed countries as well as in some developing countries[6] since 2007, using the first two licensed vaccines (a bivalent HPV16/18 vaccine and quadrivalent HPV6/11/16/18 vaccine). In late 2008, the UK began providing HPV vaccination, free at the point of delivery, routinely to 12-year-old females, and catch-up vaccination to females up to and including 17-year-olds. The bivalent vaccine was offered until September 2012 when the programme changed to offer the quadrivalent vaccine. Throughout the UK, over 80% of females eligible for routine vaccination each year have completed the three-dose course.[7–9] Three-dose coverage within the

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catch-up ages has been lower, with average coverage of 73% for individuals aged 14–15 years, and 45% for 16–17 years,[7] although this is still higher than in most other countries.[10–12]

In 2013, we reported findings from our surveillance of type-specific HPV infections in sexually active young females in England, showing evidence of substantially lower HPV16/18 prevalence in the first 4000 postvaccination period specimens tested compared with prevaccination prevalence.[13] Reductions in the prevalence of HPV16/18 following the introduction of HPV vaccination have also been shown in Australia,[14] the USA,[15–17] Scotland[18] and Sweden.[19]

Some cross-protection against non-vaccine HR-HPV types closely related to HPV16/18 has been demonstrated in clinical trials of both vaccines (specifically, HPV31, HPV33 and HPV45 for the bivalent vaccine, and HPV31 for the quadrivalent vaccine),[20–22] and has been observed for the bivalent vaccine by ongoing surveillance of young women undergoing cervical screening in Scotland.[18] Ongoing surveillance for changes in the prevalence of other non-vaccine HPV types is also prudent. These changes could result from vaccination due to cross-protection against non-vaccine HR-HPV types (ie, causing decreases in prevalence) or due to type replacement (ie, causing increases in prevalence).

We report further findings from our ongoing HPV surveillance (now over 7000 postvaccination specimens) in our high-coverage population, including changes in vaccine and non-vaccine types. We aimed to determine to what extent any such observed changes were likely to have resulted from vaccination, rather than be due to methodological reasons (eg, assay performance, unmasking), or a result of other factors such as changes in sexual behaviour over time.

METHODS

The methods of specimen selection, collection and testing, and the characteristics of the study population have been described previously.[13 23] Briefly, residual vulva-vaginal swab specimens were collected via 10 laboratories from young women aged 16–24 years undergoing chlamydia screening at general practice, community and sexual health services (CaSH, otherwise known as family planning), and youth clinics. Residual specimens were all sent for HPV testing at the Virus Reference Department laboratory at Public Health England (PHE). In England, chlamydia screening is recommended for all sexually active men and women under 25 years old annually, and on partner change, irrespective of symptoms or perceived risk. Demographic data were reported separately and linked to the specimens received at the PHE Centre for Infectious Disease Surveillance and Control. Prior to testing for HPV DNA, specimens were unlinked from any patient-identifiable data and anonymised. This study was reviewed and approved by the South East Research Ethics Committee (REC reference: 10/H1102/7). Individual patient consent was not required as this study tested anonymised specimens (with no patient-identifiable data) as part of Public Health Surveillance conducted to monitor the HPV vaccination programme.

Prevaccination-period specimens were collected between January and September 2008, prior to the introduction of the national HPV vaccination programme in England. Postvaccination-period specimens were collected between October 2010 and April 2013, and divided into two periods, 2–3 (ie, 2010–2011) and 4–5 years (ie, 2012–2013) postvaccination.

Postvaccination specimens were tested for type-specific HPV DNA to detect 13 HR types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68), five possible HR types (HPV26, 53, 70, 73 and 82) and two LR types (HPV6 and 11) using an in-house multiplex PCR and Luminex-based genotyping test with pyruvate dehydrogenase (PDH) detection for sample integrity.[24] Prevaccination specimens were tested by Hybrid Capture 2 (HC2) HPV DNA test using the Combined Probe Cocktail Method to detect HR and possible HR types (as above) and five LR types (6, 11, 42, 43 and 44) and genotyped by the Linear Array HPV Genotyping (LA) test (Roche Molecular Systems) if HC2 positive.

HPV prevalence was calculated for each individual HPV type. We also calculated combined HPV prevalence, restricted to types included in the prevaccination and postvaccination assays, for (1) any HR-HPV type; (2) the HR-HPV types included in the current vaccines: HPV16 and/or 18; (3) the additional HR-HPV types included in the nonavalent HPV vaccine[25]: HPV31, 33, 45, 52 and/or 58; (4) the HPV types for which there is some evidence of cross-protection from clinical trials: HPV31, 33 and/or 45 and (5) the non-vaccine HR-HPV types (ie, HR types not including HPV16 or 18). Changes in prevalence between the prevaccination and postvaccination (combined) periods were compared using ORs calculated using a logistic regression model. Trends over time were assessed by including three time periods (prevaccination, 2–3 years postvaccination, 4–5 years postvaccination) as an ordered continuous variable. Adjusted ORs were calculated adjusting for age, testing venue type and chlamydia positivity (as a marker for sexual behaviour). To account for the change in assay between the prevaccination and postvaccination periods, we used type-specific sensitivity and specificity estimates from a validation study (428 prevaccination specimens, retested through the postvaccination testing system[13]). The logitem command in Stata was used to adjust for the different sensitivity and specificity of the assay used in the prevaccination period. This command performs logistic regression when the binary outcome is measured with uncertainty. This adjustment did not account for the uncertainty surrounding the sensitivity and specificity estimates, hence, bootstrapping techniques were used to incorporate this additional uncertainty to the SEs of the ORs. A similar statistical model

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to adjust for assay change was not available for use with prevalence ratios (PR). However, PRs adjusted for age, testing venue type, and chlamydia positivity were also calculated using a log binomial model and the results compared with the equivalent ORs.

In order to further address concerns about changes in prevalence of HPV in the postvaccination period unrelated to vaccine introduction (eg, changes in sexual behaviour not addressed by adjustment of chlamydia positivity or residual changes in assay sensitivity), we also compared prevaccination and postvaccination type-specific prevalence when restricted to specimens with at least one HR-HPV type detected. This enabled assessment of changes in the relative, rather than absolute, frequency of specific HR types. To give a simple example, if prevaccination specimens comprised 20% HR type positivity overall (10% prevalence of type 31 and 10% prevalence of type 33), and postvaccination specimens comprised 30% HR type positivity (15% prevalence of type 31 and 15% prevalence of type 33), the absolute frequency of each type would increase postvaccination. However, the relative prevalence of each type within the HR-HPVs positives would remain the same.

Vaccination coverage by age and time period was derived from published data.[7–9 26]

RESULTS

Demographics and characteristics

Results were analysed from 2354 prevaccination specimens and 7321 postvaccination specimens: 3602 (49.2%) from 2–3 years postvaccination, and 3719 (50.8%) from 4–5 years postvaccination. The characteristics of study participants were similar in the prevaccination and postvaccination periods (table 1), except there were more specimens from women of non-white ethnic groups, and fewer specimens collected from youth clinics in the postvaccination collection (7.3% vs 17.6%, and 24.1% vs 3.1%, respectively). Furthermore, two laboratories, Leeds and Lewisham, included in only the postvaccination period, had notably higher chlamydia positivity rates than the other laboratories (22.4% and 8.4%, respectively). Data from these laboratories were excluded in sensitivity analyses. In the postvaccination period, the estimated vaccination coverage in the surveillance population, based on nationally reported data, was 67.2%, 30.7% and 0.6% for individuals aged 16–18, 19–21 and 22–24 years, respectively (table 2 for estimates by time period).

HR HPV16 and/or 18 infection

In the youngest age group (16–18 years), the prevalence of HPV16/18 was 17.6% in the prevaccination period compared to 8.5% in the period 2–3 years postvaccination, and 4.0% 4–5 years postvaccination (p value for trend <0.001; table 2 and figure 1). This corresponds to an overall reduction of 66% comparing the prevaccination prevalence to the combined postvaccination prevalence. A trend was also seen in individuals aged 19–21 years with a prevaccination prevalence of 16.9% compared to 14.2% in the period 2–3 years postvaccination, and 8.7% 4–5 years postvaccination (p value for trend <0.001; combined reduction between the prevaccination and postvaccination periods of 31%). However, there was no decrease in the prevalence of HPV16 and/or HPV18 in the oldest age group, who were largely unvaccinated. There was a slight decrease in HPV18 infection in the oldest age group, but this difference was no longer seen once adjustment was made for changes in population and HPV assay (data not shown).

The adjusted ORs for the postvaccination periods (combined) compared with the prevaccination period were 0.3 (95% CI 0.2 to 0.4), 0.6 (95% CI 0.5 to 0.9) and 1.1 (95% CI 0.8 to 1.7) for individuals aged 16–18, 19–21 and 22–24 years, respectively (table 3).

HR HPV31, 33 and/or 45 infection (cross-protective HPV types)

The prevalence of HPV31, 33 and/or 45 among the individuals aged 16–18 years was 8.4% in the prevaccination period, 6.9% in the period 2–3 years postvaccination, and 5.8% 4–5 years postvaccination. After adjusting for demographics and the change in HPV assay, the adjusted OR postvaccination (combined) was 0.9 (95% CI 0.5 to 1.5), p value=0.58 (tables 2 and 3). The prevalence of HPV31 in this age group reduced from 3.7% in the prevaccination period to 0.9% in the combined postvaccination period. This reduction did not reach statistical significance after adjustment for the known population changes and the assay change (adjusted OR 0.4 (95% CI 0.2 to 2.9), p value=0.21): there was no evidence of a reduction in the overall prevalence of HPV33 or of HPV45. Among women aged 16–18 years with at least one non-vaccine HR-HPV type detected, the prevalence of HPV31/33/45 was 48% lower in the period 4–5 years postvaccination compared to the prevaccination period, with a reduction from 14.9% to 3.7% for HPV31, 9.6% to 7.9% for HPV33, and 11.5% to 6.5% for HPV45 (see online supplementary table S1). In the older age groups, with lower vaccination coverage, there was no evidence of a reduction in these three HPV types between the prevaccination and postvaccination periods.

Non-vaccine HR-HPV types

There was an increase in the prevalence of non-vaccine HR-HPV types between the prevaccination and postvaccination periods at all ages (24.9–33.7%, 26.9–39.6% and 26.4–32.9% for individuals aged 16–18, 19–21 and 22–24 years, respectively). After adjustment for age, venue type, chlamydia positivity and the change in assay, the adjusted ORs comparing the prevaccination and postvaccination prevalence were 1.3 (1.0 to 1.7), 1.5 (1.1 to 2.0) and 1.2 (0.9 to 1.6) for individuals aged 16–18, 19–21 and 22–24 years, respectively (table 3). There was also evidence for increases in the prevalence of the

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Table 1 Characteristics of women included in the prevaccination and postvaccination surveys

	Prevaccination (2008) (n=2354)	Postvaccination (2010–2011) (n=3602)	Postvaccination (2012–2013) (n=3719)
Number of samples by laboratory			
North West (Aintree)	472 (20.1%)	170 (4.7%)	350 (9.4%)
Yorkshire and The Humber (Leeds)	–	620 (17.2%)	883 (23.7%)
West Midlands (Stoke)	260 (11.0%)	259 (7.2%)	219 (5.9%)
East of England (Norfolk and Norwich)	759 (32.2%)	222 (6.2%)	123 (3.3%)
East of England (Cambridge)	–	345 (9.6%)	588 (15.8%)
South East (East Kent)	–	563 (15.6%)	935 (25.1%)
South East (Portsmouth)	–	81 (2.2%)	–
South West (Cornwall)	473 (20.1%)	439 (12.2%)	453 (12.2%)
London (University College London)	390 (16.6%)	476 (13.2%)	–
London (Lewisham)	–	427 (11.9%)	168 (4.5%)
Age, years (data completeness)	(100%)	(100%)	(100%)
16–18	1047 (44.5%)	933 (25.9%)	1063 (28.6%)
19–21	804 (34.2%)	1463 (40.6%)	1310 (35.2%)
22–24	503 (21.4%)	1206 (33.5%)	1346 (36.2%)
Ethnicity (data completeness)	(88%)	(76%)	(62%)
White	1924 (92.7%)	2119 (77.1%)	2058 (88.6%)
Black	93 (4.5%)	392 (14.3%)	158 (6.8%)
Asian	25 (1.2%)	75 (2.7%)	46 (2.0%)
Other	34 (1.6%)	144 (5.2%)	46 (2.0%)
Sample collection venue (data completeness)	(100%)	(100%)	(100%)
General practice	608 (25.8%)	1085 (30.1%)	1257 (33.8%)
Family planning (Community Sexual Health Services)	1179 (50.1%)	2429 (67.4%)	2320 (62.4%)
Youth clinic	567 (24.1%)	88 (2.4%)	142 (3.8%)
2+ sexual partners in the previous 12 months (data completeness)	53.6% (81%)	46.6% (45%)	48.6% (31%)
New sexual partner in the previous 3 months (data completeness)	48.1% (81%)	48.1% (47%)	51.3% (32%)
Chlamydia positivity (data completeness)	8.9% (99%)	7.3% (99.8%)	8.5% (100%)
Chlamydia positivity (excluding Leeds and Lewisham) (data completeness)	NA	4.7% (99.8%)	2.7% (100%)
Proportion eligible for HPV vaccination	0.0%	45.6%	61.3%
Estimated vaccination coverage	0.0%	24.3%	35.8%

NA, not applicable.

additional nonavalent HR-HPV types in the age group of 19–21 years. Adjusted ORs were 1.2 (0.9 to 1.7), 1.5 (1.1 to 2.2) and 1.3 (0.9 to 2.0), respectively. This increase in non-vaccine HR-HPV types was only seen between the prevaccination and postvaccination combined periods. Within the postvaccination period, there was no evidence of a change in the prevalence of these HR-HPV types over time (table 2).

The type-specific prevalence of HPV58 was similar in the prevaccination and postvaccination period for all age groups. However, there was an increase in the prevalence of HPV52 even after adjustment (adjusted OR 1.7 (1.0 to 3.2) and 2.4 (1.4 to 4.7), respectively) for individuals aged 16–18 and 19–21 years, and a borderline increase for individuals aged 22–24 years (1.6 (0.9 to 3.6)).

LR HPV6 and/or 11 infection

Similar to the non-vaccine HR types, there was a significant increase in the prevalence of HPV6/11 in the postvaccination period among women aged 16–18 years (5.8% prevaccination vs 8.3% postvaccination; adjusted OR 1.9 (1.1 to 3.4)). There was also a slight increase in the LR types for individuals aged 19–21 years (5.8% vs 7.6%, respectively; adjusted OR 1.4 (0.8 to 2.6)) although after adjustment for age, venue type, chlamydia positivity and the change in assay, this was not significant (p=0.15). There was no evidence of a change in the prevalence of HPV6/11 in the older age group (22–24-years, 4.4% prevaccination vs 4.3% postvaccination; adjusted OR 1.2 (0.6 to 4.1)).

Repeating analyses using PRs instead of ORs (adjusted for all factors except for assay change) gave very similar results for all HPV types (results not shown).

DISCUSSION

This surveillance of young sexually active women undergoing chlamydia screening has demonstrated continuing reductions in the prevalence of the HPV vaccine types following the introduction of a high-coverage national

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Table 2 Estimates of prevalence of HPV types by age in prevaccination and postvaccination periods

HPV type	Prevaccination prevalence (%) 2008 (95% CI) n=2354	Postvaccination prevalence (%) 2010–2011 (95% CI) n=3602	Postvaccination prevalence (%) 2012–2013 (95% CI) n=3719	p-value for trend
16–18 years				
(Estimated HPV16/18 vaccination coverage)	(0%)	(60.2%)	(73.4%)	
Any high-risk HPV	32.6 (29.7 to 35.4)	37.6 (34.5 to 40.7)	35.4 (32.5 to 38.3)	0.188
Any non-vaccine high-risk HPV	24.9 (22.3 to 27.6)	34.2 (31.1 to 37.2)	33.2 (30.4 to 36.0)	<0.001
Vaccine HPV types				
HPV16 and/or 18	17.6 (15.3 to 19.9)	8.5 (6.7 to 10.3)	4.0 (2.8 to 5.1)	<0.001
HPV16	11.9 (10.0 to 13.9)	6.8 (5.1 to 8.4)	3.0 (2.0 to 4.0)	<0.001
HPV18	7.8 (6.2 to 9.5)	2.8 (1.7 to 3.8)	1.1 (0.5 to 1.8)	<0.001
Nonavalent HPV types*				
HPV31/33/45/52/58	14.5 (12.4 to 16.7)	17.7 (15.2 to 20.1)	14.9 (12.7 to 17.0)	0.835
HPV31/33/45	8.4 (6.7 to 10.1)	6.9 (5.2 to 8.5)	5.8 (4.4 to 7.2)	0.021
HPV31	3.7 (2.6 to 4.9)	0.5 (0.1 to 1.0)	1.2 (0.6 to 1.9)	<0.001
HPV33	2.4 (1.5 to 3.3)	3.5 (2.3 to 4.7)	2.6 (1.7 to 3.6)	0.739
HPV45	2.9 (1.9 to 3.9)	2.9 (1.8 to 4.0)	2.2 (1.3 to 3.0)	0.314
HPV52	4.0 (2.8 to 5.2)	8.6 (6.8 to 10.4)	6.4 (4.9 to 7.9)	0.027
HPV58	3.7 (2.6 to 4.9)	4.0 (2.7 to 5.2)	3.9 (2.7 to 5.0)	0.875
19–21 years				
(Estimated HPV16/18 vaccination coverage)	(0%)	(21.4%)	(41.1%)	
Any high-risk HPV	34.3 (31.0 to 37.6)	45.9 (43.4 to 48.5)	44.2 (41.5 to 46.9)	<0.001
Any non-vaccine high-risk HPV	26.9 (23.8 to 29.9)	39.1 (36.6 to 41.6)	40.2 (37.5 to 42.8)	<0.001
Vaccine HPV types				
HPV16 and/or 18	16.9 (14.3 to 19.5)	14.2 (12.4 to 16.0)	8.7 (7.2 to 10.2)	<0.001
HPV16	12.6 (10.3 to 14.9)	11.1 (9.5 to 12.7)	7.5 (6.1 to 8.9)	<0.001
HPV18	6.5 (4.8 to 8.2)	3.8 (2.8 to 4.7)	1.7 (1.0 to 2.4)	<0.001
Nonavalent HPV types*				
HPV31/33/45/52/58	15.2 (12.7 to 17.7)	21.2 (19.1 to 23.3)	20.2 (18.1 to 22.4)	0.015
HPV31/33/45	8.3 (6.4 to 10.2)	8.7 (7.2 to 10.1)	8.8 (7.2 to 10.3)	0.736
HPV31	4.7 (3.3 to 6.2)	2.3 (1.5 to 3.0)	2.7 (1.8 to 3.5)	0.019
HPV33	2.0 (1.0 to 3.0)	2.9 (2.0 to 3.7)	3.4 (2.4 to 4.4)	0.058
HPV45	2.6 (1.5 to 3.7)	3.7 (2.7 to 4.7)	3.2 (2.3 to 4.2)	0.581
HPV52	4.1 (2.7 to 5.5)	10.0 (8.5 to 11.6)	10.3 (8.7 to 12.0)	<0.001
HPV58	5.0 (3.5 to 6.5)	4.6 (3.6 to 5.7)	4.0 (2.9 to 5.0)	0.256
22–24 years				
(Estimated HPV16/18 vaccination coverage)	(0%)	(0%)	(1.1%)	
Any high-risk HPV	32.8 (28.7 to 36.9)	40.4 (37.6 to 43.2)	42.4 (39.8 to 45.1)	0.001
Any non-vaccine high-risk HPV	26.4 (22.6 to 30.3)	32.0 (29.4 to 34.6)	33.7 (31.1 to 36.2)	0.007
Vaccine HPV types				
HPV16 and/or 18	15.3 (12.2 to 18.5)	16.5 (14.4 to 18.6)	16.1 (14.2 to 18.1)	0.790
HPV16	10.9 (8.2 to 13.7)	14.7 (12.7 to 16.7)	13.6 (11.8 to 15.4)	0.334
HPV18	5.8 (3.7 to 7.8)	2.7 (1.7 to 3.6)	3.0 (2.1 to 3.9)	0.019
Nonavalent HPV types*				
HPV31/33/45/52/58	16.7 (13.4 to 20.0)	18.4 (16.2 to 20.6)	21.1 (18.9 to 23.3)	0.020
HPV31/33/45	8.9 (6.4 to 11.4)	7.9 (6.4 to 9.4)	10.1 (8.5 to 11.7)	0.196
HPV31	3.2 (1.6 to 4.7)	2.5 (1.6 to 3.4)	2.7 (1.9 to 3.6)	0.770
HPV33	2.6 (1.2 to 4.0)	2.1 (1.3 to 2.9)	3.5 (2.5 to 4.5)	0.111
HPV45	4.2 (2.4 to 5.9)	3.6 (2.6 to 4.7)	4.2 (3.1 to 5.2)	0.837
HPV52	5.2 (3.2 to 7.1)	8.6 (7.0 to 10.2)	9.7 (8.1 to 11.2)	0.005
HPV58	3.0 (1.5 to 4.5)	3.2 (2.2 to 4.1)	3.4 (2.4 to 4.4)	0.605

*Defined as the additional HPV types included in the nonavalent vaccine (31, 33, 45, 52 and 58).
HPV, human papillomavirus.

HPV vaccination programme as well as some evidence of overall reductions in HPV31 (the closely related HPV type with strongest evidence of cross-protection from the bivalent vaccine clinical trials[22]). Encouragingly, these reductions are more marked in the later postvaccination period with higher estimated vaccination coverage. Use of bootstrapping techniques to account for the uncertainty of the specificity and sensitivity estimates from the validation study provided conservative estimates with wider CIs. Once we accounted for this additional

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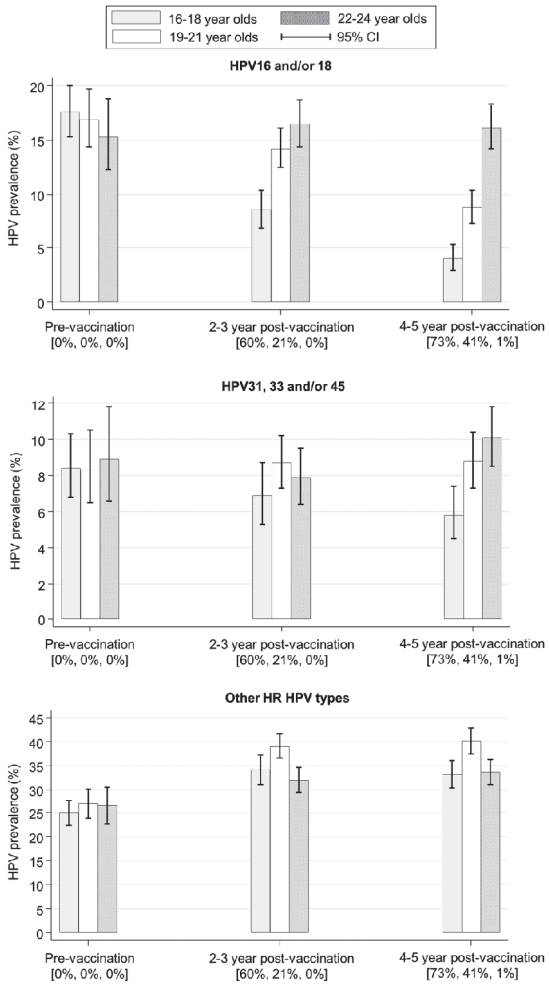

Figure 1 Prevaccination and postvaccination prevalence of human papillomavirus (HPV) types by age. Percentages in square brackets represent estimated three-dose HPV vaccination coverage for individuals aged 16–18, 19–21 and 22–24 years, respectively. HR, high risk.

uncertainty, the reduction in the prevalence of HPV31 postvaccination no longer reached statistical significance.

The percentage reductions between the postvaccination and prevaccination periods among the youngest two age groups were very similar to the estimated vaccine coverage. If all the reduction in prevalence was due to a direct effect of vaccination, this would be consistent with close to 100% vaccine effectiveness. Such high vaccine effectiveness is unlikely given that women included in this surveillance were largely vaccinated as part of the catch-up programme, and almost certainly some of those vaccinated would have had an existing HPV infection. These high reductions could be partly explained by the fact that nationally published data that was used to estimate vaccination coverage is based on reported data on vaccination administration. A recent study of serological markers has suggested that these administration data may be under-reporting HPV vaccinations among women eligible for vaccination as part of the catch-up programme (D Mesher, E Stanford, J White, *et al*. HPV serology testing confirms high HPV immunisation coverage in England. Submitted for publication 2015). This would mean that the vaccination coverage we had estimated for our surveillance population would have been a slight under-estimate. This would be more consistent with the relatively high overall reductions in HPV16/18 we observed although it is most likely that these are due to a combination of both higher vaccination coverage and some herd protection effect.

This surveillance makes use of a large sample of residual specimens taken for chlamydia screening and tested anonymously for HPV-DNA infection. Young women attending for chlamydia screening have higher risks of chlamydia, and therefore, probably for HPV infection, than the general population. The reductions in the HPV vaccine types (HPV16/18) observed here, therefore, reassures that benefits of HPV vaccination have not been inequitably biased to lower risk individuals.

The observation that the reductions in HPV16/18 were only seen in the age groups eligible for national HPV vaccination, and reduced further in the later postvaccination period (ie, were proportionate to estimated vaccination coverage), strongly suggests that the changes seen are attributable to vaccination.

If increases in the other HR-HPV types were restricted to the younger age groups, or were greater in the later postvaccination period with higher vaccination coverage, then this could raise suspicion of potential type replacement. However, the increases seen in the non-vaccine HPV types were seen in all age groups, including the older unvaccinated women, which suggest that these increases are unlikely to be due to type replacement, and are more likely a result of limitations in our study. First, comparison of HPV prevalence between the prevaccination and postvaccination periods were adjusted for age, venue type and chlamydia positivity (as a marker of sexual behaviour). However, other changes in the population characteristics (or sexual behaviour not captured by chlamydia positivity) may have resulted in a change in prevalence of the non-vaccine HR-HPV types. If women in the postvaccination period were at a higher risk of HPV infection then this could have underestimated the potential effect of HPV vaccination on the HPV vaccine types. Analyses restricted to women with at least one HR-HPV type show larger declines for HPV31 and evidence of a reduction in HPV45 which would support this hypothesis. Furthermore, these analyses restricted to HR-HPV-positive specimens show little difference in relative prevalence of HPV52 or 58, which strengthens our conclusion that these increases are probably not due to type replacement. Second, there

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Table 3 Prevalence and OR of HPV infection in the postvaccination period compared to prevaccination, by age group

	Prevaccination: n (%)	Postvaccination: n (%)	OR (95% CI)	Adjusted OR* (95% CI)
16–18 years				
(Estimated HPV16/18 vaccination coverage)	(0%)	(67.2%)		
HPV16/18 with or without other HR types	184 (17.6%)	121 (6.1%)	0.3 (0.2 to 0.4)	0.3 (0.2 to 0.4)
HPV16/18 alone	80 (7.6%)	55 (2.8%)	0.3 (0.2 to 0.5)	0.5 (0.3 to 1.3)
Non-vaccine HR type(s) with or without HPV16/18	261 (24.9%)	672 (33.7%)	1.5 (1.3 to 1.8)	1.3 (1.0 to 1.7)
HPV31/33/45	88 (8.4%)	126 (6.3%)	0.7 (0.6 to 1.0)	0.9 (0.5 to 1.5)
HPV31/33/45/52/58	152 (14.5%)	323 (16.2%)	1.1 (0.9 to 1.4)	1.2 (0.9 to 1.7)
19–21 years				
(Estimated HPV16/18 vaccination coverage)	(0%)	(30.7%)		
HPV16/18 with or without other HR types	136 (16.9%)	322 (11.6%)	0.6 (0.5 to 0.8)	0.6 (0.5 to 0.9)
HPV16/18 alone	60 (7.5%)	153 (5.5%)	0.7 (0.5 to 1.0)	1.2 (0.6 to 4.5)
Non-vaccine HR type(s) with or without HPV16/18	216 (26.9%)	1098 (39.6%)	1.8 (1.5 to 2.1)	1.5 (1.1 to 2.0)
HPV31/33/45	67 (8.3%)	242 (8.7%)	1.1 (0.8 to 1.4)	1.3 (0.8 to 2.6)
HPV31/33/45/52/58	122 (15.2%)	575 (20.7%)	1.5 (1.2 to 1.8)	1.5 (1.1 to 2.2)
22–24 years				
(Estimated HPV16/18 vaccination coverage)	(0%)	(0.6%)		
HPV16/18 with or without other HR types	77 (15.3%)	416 (16.3%)	1.1 (0.8 to 1.4)	1.1 (0.8 to 1.7)
HPV16/18 alone	32 (6.4%)	219 (8.6%)	1.4 (0.9 to 2.0)	2.5 (1.2 to 329.2)
Non-vaccine HR type(s) with or without HPV16/18	133 (26.4%)	839 (32.9%)	1.4 (1.1 to 1.7)	1.2 (0.9 to 1.6)
HPV31/33/45	45 (8.9%)	231 (9.1%)	1.0 (0.7 to 1.4)	1.2 (0.7 to 2.5)
HPV31/33/45/52/58	84 (16.7%)	506 (19.8%)	1.2 (1.0 to 1.6)	1.3 (0.9 to 2.0)

*Adjusted for age, venue type, chlamydia positivity and change in HPV assay between prevaccination and postvaccination period.
HPV, human papillomavirus.

was a change in the assay used between the postvaccination and prevaccination periods, but no change in the assay used throughout the postvaccination period; hence, continued reductions in the vaccine HPV types within the postvaccination period cannot be affected by this. However, it was necessary to adjust ORs comparing the prevaccination and postvaccination periods for the different assays used. Finally, broad-spectrum assays, such as those used in our study, can lack sensitivity to detect individual HPV types at low copy number in the presence of other HPV types. Therefore, the decrease in multiple HPV infections due to the reduction in HPV16 and 18 following vaccination could lead to an apparent, artificial increase in the prevalence of certain non-vaccine HPV types (ie, unmasking). Given that the increases in certain HPV types were apparent between the prevaccination and postvaccination periods, but remained relatively stable within the postvaccination period, this suggests that unmasking is not playing a huge role in these increases. However, while adjustment was made for the change in assay between the two periods, to what extent increases in non-vaccine types are due to temporal changes, changes in the population undergoing chlamydia screening, or changes in the detection accuracy of assays, is still somewhat unclear.

In England, the quadrivalent vaccine was introduced to the national HPV immunisation programme from 2012 as part of routine vaccination of 12-year-old girls. At the time this surveillance was conducted, the oldest women vaccinated with the quadrivalent vaccine as part of the national programme would have been 14 years old, hence, too young to be included in this surveillance (conducted among individual aged 16–24 years). Therefore, all women included in this surveillance who were vaccinated as part of the national immunisation programme would have received the bivalent HPV vaccine. We were unable to link these specimens to individual HPV vaccination status, and coverage estimates were derived from published data. This meant that we considered population-level impact of HPV vaccination rather than direct calculation of vaccine effectiveness. Our findings of reductions in the prevalence of the HPV vaccine types are consistent with surveillance conducted in other countries, although changes in the prevalence of non-vaccine HR-HPV types varied.[27] Tabrizi *et al*[14] showed a 77% reduction in the prevalence of HPV vaccine types among young women attending for a Pap test in Australia. In the USA, reductions in the prevalence of HPV vaccine types were 56% in individuals aged 14–19 years, despite a low self-reported vaccination coverage (34% with one or more doses).[17] In Sweden, surveillance also among women attending for chlamydia screening found a reduction of 42% in HPV16 and 46% in HPV18 among females aged 13–22 years, and also a slight increase in HPV52 and 56.[19] In Scotland, where cervical screening is offered from age 20 years, a 54% reduction in the vaccine types has been shown in individuals aged 20 years, as well as a 48% reduction in the cross-protection types HPV31, 33 and 45.[18]

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We have analysed HPV type-specific prevalence among almost 10,000 women over a period of 5 years. These data provide clear evidence of a reduction in the HPV vaccine types, and a suggestion of a reduction in HPV31, a closely related HPV type, since the introduction of the HPV immunisation programme in England. This will both inform future decisions regarding HPV vaccination in England and be of interest to other countries seeking to monitor the impact of HPV vaccination.

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Contributors This surveillance was initiated and designed by KS. DM and KS were responsible for the sample collection and data management. SB and KP performed the HPV testing. DM conducted the statistical analysis. SLT contributed to the data analysis and interpretation. DM, KS and SLT wrote the first draft of the manuscript. All authors contributed to and approved the final draft.

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RESEARCH PAPER COVER SHEET

PLEASE NOTE THAT A COVER SHEET MUST BE COMPLETED FOR EACH RESEARCH PAPER INCLUDED IN A THESIS.

SECTION A – Student Details

Student	David Mesher
Principal Supervisor	Sara Thomas
Thesis Title	Assessment of the population-level impact of a high coverage HPV immunisation programme in young females

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

Where was the work published?			
When was the work published?			
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
Have you retained the copyright for the work?*	Choose an item.	Was the work subject to academic peer review?	Choose an item.

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SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	The Journal of Infectious Diseases
Please list the paper's authors in the intended authorship order:	David Mesher, Kavita Panwar, Sara L Thomas, Claire Edmundson, Yoon Hong Choi, Simon Beddows, Kate Soldan
Stage of publication	**Undergoing revision**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	The post-vaccination surveillance (commencing in October 2010) was initiated and designed by Kate Soldan. Since October 2011, I was responsible for liaising with local laboratories providing samples for the post-vaccination surveillance. I was also

	responsible for all data collection and management. I performed the data linkage with the Chlamydia Testing Activity Dataset (CTAD). I designed and established the collection of HPV vaccination records and I performed the data linkage of these records. I performed anonymisation of patient identifiable data and liaised with the PHE laboratory to inform them when samples had been anonymised and could be tested. Kavita Panwar and Simon Beddows performed the laboratory testing. I conducted all data management and statistical analyses with advice from Sara Thomas and Kate Soldan. I wrote the first draft of the manuscript which was commented on by all authors. This paper was peer reviewed and I incorporated suggestions from reviewers and responded to their comments, with input from other authors.

Student Signature: [redacted] **Date:** 12/04/2018

Supervisor Signature: [redacted] **Date:** 13/04/18

Title: The impact of the national HPV vaccination programme in England using the bivalent HPV vaccine: surveillance of type-specific HPV in young females, 2010-2016

Authors: David Mesher[1,2], Kavita Panwar[3], Sara L Thomas[2], Claire Edmundson [1], Yoon Hong Choi[4], Simon Beddows[3], Kate Soldan[1]

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Abstract

Background: The National HPV Immunisation Programme was introduced in England in September 2008 using the bivalent vaccine.

Methods: We collected residual vulva-vaginal swab (VVS) specimens from 16-24 year old women attending for chlamydia screening between 2010 and 2016 and tested them for HPV DNA. We compared changes in type-specific (vaccine and non-vaccine) HPV prevalence over time and the association with vaccination coverage. For women with known vaccination status, vaccine-effectiveness was estimated.

Results: HPV DNA testing was completed for 15,459 specimens. The prevalence of HPV16/18 decreased between 2010/11 and 2016 from 8.2% to 1.6% in 16-18 year olds and from 14.0% to 1.6% in 19-21 year olds. Declines were also seen for HPV31/33/45 (6.5% to 0.6% for 16-18 year olds and 8.6% to 2.6% for 19-21 year olds). Vaccine-effectiveness for HPV16/18 was 82.0% (95% CI: 60.6-91.8) and for HPV31/33/45 was 48.7% (95% CI: 20.8-66.8). Prevalence of HPV16/18 was compared to findings in 2007-8 (pre-vaccination) and to predictions from Public Health England's mathematical model.

Discussion: Eight years after the introduction of a national HPV vaccination programme, substantial declines have occurred in HPV16/18 and in HPV31/33/45. The prevalence of other high-risk HPV types has not changed.

Introduction

Cervical cancer is caused by persistent human papillomavirus (HPV) infection[127]. In the UK, a national programme was introduced in 2008 to offer HPV vaccination routinely to 12-13 year olds, and offer catch-up vaccination to girls up to the age of 18 years old. The UK national programme initially used the bivalent HPV vaccine (Cervarix®). This vaccine has demonstrated high efficacy against two high-risk (HR) HPV types, HPV16 and HPV18[128, 129], which have been shown to be responsible for around 70% of cervical cancers worldwide[130] and around 80% of cancers in the UK[131]. In 2012, the UK programme changed to use the quadrivalent vaccine (Gardasil®) which additionally offers high efficacy against two low-risk (LR) types, HPV6 and HPV11[132, 133], which cause the majority of genital warts[134]. HPV vaccination coverage in England has been high with over 80% of 12-13 year olds receiving the full course (3-doses prior to September 2014, 2-doses since). Coverage within the catch-up cohorts has been lower and more variable (ranging from 39% to 76%)[78]. A reduction in cervical cancer incidence is not expected for some years given the time interval between vaccination and the age of most cervical cancer diagnoses; thus, a reduction in the prevalence of HPV infection can provide an early indication of the effectiveness of the vaccination programme. Data from several countries have already demonstrated reductions in vaccine types and HPV31, 33 and 45 since the introduction of national HPV vaccination programmes[135, 136].

In England, we utilise residual genital specimens, collected for chlamydia screening, for national HPV surveillance. We previously reported results showing lower prevalence of HPV16/18 in the period after vaccination compared to before vaccination was introduced, as well as some evidence of a reduction in HPV31, likely due to cross-protection[19, 90, 126]. The latest of these reports[90] compared type-specific prevalence for 2,354 specimens collected in 2008 to 7,321 specimens

collected from 2010 to 2013. These findings largely related to females eligible for catch-up vaccination. We now report an extension of these results with specimens collected to December 2016, including females eligible for routine vaccination. We also include results stratified by HPV vaccination status.

We report trends in HPV16/18 prevalence since HPV vaccination began in England and vaccine-effectiveness estimates for the bivalent HPV vaccine. We compare findings to predictions from transmission dynamic model that informed the vaccination policy[137]. Evidence for herd protection effects and for cross-protection against non-vaccine HPV types is also explored.

Methods

Specimen and data collection

The methods for collection and testing of specimens and linkage with data have been described elsewhere[90, 126]. In brief, residual vulva-vaginal swab (VVS) specimens were collected from 16-24 year old women attending for chlamydia screening at general practices (GP), community and sexual health services (CaSH) or youth clinics. Residual specimens were collected from ten laboratories where chlamydia testing was performed (University Hospital Aintree [Aintree]; Leeds Teaching Hospitals [Leeds]; University Hospital of North Staffordshire [Stoke]; Norfolk and Norwich University Hospital [Norfolk and Norwich]; Addenbrooke's Hospital [Cambridge]; East Kent Hospitals University [East Kent]; Queen Alexandra Hospital [Portsmouth]; Royal Cornwall Hospital [Cornwall]; University College London Hospital [UCL]; and University Hospital Lewisham [Lewisham]). Specimens were sent to the Virus Reference Department (VRD) at Public Health England (PHE) for HPV testing. Demographic data were reported to PHE separately, either by the clinic (prior to 2012) or laboratory performing the chlamydia testing (from 2012 onwards). Demographic data were linked to specimens using a unique study

number and, once linked, all other identifiable data were removed prior to HPV testing. Laboratories were asked to submit a specified number of specimens for each year/age-group to meet target sample sizes. Since 2015, samples were only requested from 16-20 year olds who would have largely been in routine vaccination cohorts. As these samples were collected for routine public health surveillance conducted to monitor the HPV vaccination programme, individual patient consent was not required. PHE has permission to handle these data under section 251 of the UK National Health Service Act of 2006 (previously section 60 of the Health and Social Care Act of 2001), which was renewed annually by the ethics and confidentiality committee of the National Information Governance Board until 2013. Since then, the power of approval of public health surveillance activity has been granted directly to PHE.

The present analysis included women who were eligible for bivalent HPV vaccination as part of the national programme (i.e. born on or after 1st September 1990) as well as some too old to have been eligible. The latter were included to provide a comparison to vaccine-eligible women, and allow observation of changes in HPV prevalence due to herd protection. The number of women eligible for the quadrivalent vaccine in the national programme (i.e. born on or after 1st September 1999) was very small; these women were excluded. Since 2014, HPV vaccination status has been retrospectively sought for specimens collected from women eligible for vaccination. Two different methods have been used to obtain these data: data obtained from laboratories from the chlamydia-test request form, and data obtained by linkage with local Child Health Information Service (CHIS) Systems. The latter method could only be used if (i) chlamydia testing laboratories provided NHS number, and (ii) local CHIS system was able to conduct the linkage. One laboratory used the former method (Lewisham) and for four we used the latter method (Cambridge, Cornwall, Portsmouth and Stoke). The completion of linkage to vaccine

status varied greatly across these laboratories. For the remaining five laboratories (Aintree, East Kent, Leeds, Norfolk and Norwich and UCL) HPV vaccination status data were unavailable. Data collected from CHIS included the number of doses given and dates of each dose. Data collected from the chlamydia-test request form was self-reported vaccination status and did not include information on the dates of doses. We assumed that vaccinated women with an unknown number of doses (126/1952; 6.5%) were fully vaccinated; this was explored further in sensitivity analyses.

Vaccination coverage for all women, stratified by year and age group, was estimated by combining individual-level vaccination coverage (for those whom vaccination status could be obtained) with the published national vaccination coverage (for those whom vaccination status could not be obtained).

HPV testing

Eligible specimens were tested using an in-house multiplex PCR and Luminex-based genotyping test for 13 HR HPV types (HPV16/18/31/33/35/39/45/51/52/56/58/59/68), five possible HR types (HPV26/53/70/73/82) and two LR types (HPV6/11)[116]. An inadequate result was given if the samples were negative for both HPV and the housekeeping gene, pyruvate dehydrogenase (PDH)[116].

Statistical analysis

We calculated the HPV prevalence and 95% confidence intervals for three age-groups (16-18, 19-21 and 22-24 year olds) and four time periods post-vaccination (2010-2011, 2012-2013, 2014-2015 and 2016). This was calculated for; (i) individual HPV types, (ii) any HR HPV type (HPV16/18/31/33/35/39/45/51/52/56/58/59/68); (iii) HPV vaccine types (HPV16/18); (iv) HPV31/33/45 with cross-protective efficacy established by clinical trials[73, 76]; (v) any other HR HPV type

(HPV35/39/51/52/56/58/59/68)[2], and (vi) additional HR HPV types included in the nonavalent HPV vaccine (HPV31/33/45/52/58). Changes in prevalence over time were compared using a log-binomial regression model, adjusted for age, testing venue and chlamydia (as a marker for sexual behaviour). For HPV16/18, previously published prevalence estimates for 2007/2008 (i.e. prior to HPV vaccination) were included in trend analyses[19]. For all other HPV types, trend analysis considered changes within the post-vaccination period only, due to differences in detection rates for certain types with the different assay used in the pre- vaccination period. This trend analysis approximated the effect of increasing vaccination coverage on HPV prevalence (i.e. what are the changes in HPV prevalence as vaccination coverage increases over time). However, to further quantify the association between HPV vaccination coverage and changes in post-vaccination HPV prevalence for all women across all years (2010-2016), we included a continuous variable in the regression model with the estimated HPV vaccination coverage (as a proportion). This allowed us to estimate an adjusted prevalence ratio for HPV infection comparing a population with no female vaccination (coverage=0) with a fully vaccinated population (coverage=1). Percentage declines in HPV16 and HPV18 within the post-vaccination period were also plotted against predictions from a previously published model[137] . To do this, we input the published vaccination coverage[78] into this transmission dynamic model and calculated the percentage reductions for HPV16 and HPV18 from 100 best fitting scenarios to the pre-vaccination prevalence[137]. The median of these 100 outputs were then calculated along with minimum and maximum values to give a range.

Risk ratios (RRs) comparing HPV prevalence in vaccinated vs. unvaccinated women (for all years combined, 2010-2016) were calculated using a log-binomial regression model (adjusted (aRR) for age, testing venue type and chlamydia positivity). Vaccine-effectiveness was calculated as 1-aRR. These analyses were stratified by

age at vaccination (females offered vaccination at 12-15 years old vs. females offered vaccination at 16-17 years old).

Sensitivity analysis

In sensitivity analyses, we calculated vaccine-effectiveness for women known to have had all vaccine doses in the recommended time interval. Specifically, we excluded women with (i) unknown number of doses (n=126), and (ii) vaccinated outside of the recommended dose schedule for the bivalent vaccine (n=307).

Results

Participant characteristics

A total of 15,459 specimens were included in this analysis : 4,044 samples collected in 2010-2011, 7,253 in 2012-2013 2,737 in 2014-15 and 1,425 in 2016 (Table 1).

The distribution of specimens by ethnicity has remained relatively stable with a slightly higher proportion of black women in 2010-2011 and an increasing proportion with missing ethnicity in more recent years as data have been sourced from laboratories rather than clinics. Over time, the proportion of samples from women attending GPs has increased, and the proportion from CaSH and youth clinics has decreased. Chlamydia positivity has decreased over time from just over 8% in 2010-11 to around 6% in the later periods, likely reflecting changes in the population undergoing chlamydia screening. Changes in distributions of samples by ethnicity, recruitment venue and chlamydia positivity were similar within each age-group (data not shown).

As expected, the proportion of women who had been eligible for vaccination increased over time (Table 1). This was partly due to a higher proportion of women in later years having been offered the vaccine at younger ages with higher national coverage but also due to a change in our sampling as we only requested samples

from 16-20 year olds from 2015 onwards. Of 11,199 women eligible to receive the HPV vaccine as part of the national programme, 2,318 (20.7%) had a known vaccination status (1,924 (84%) from Cornwall laboratory). Of these, 2,159 (93.1%) were obtained from CHIS records and 159 (6.9%) were self-reported. Among these women, full-course coverage was 58.2%, 75.7%, 85.4% and 86.7% in 2010-2011, 2012-2013, 2014-2015 and 2016 respectively.

Characteristics of women who would have been eligible for vaccination in the national programme are given in Table 2, stratified by vaccination status (unvaccinated, vaccinated or unknown status). Among unvaccinated women, there was a slightly higher chlamydia positivity, a higher proportion of black women and higher proportion of samples from family planning clinics compared to vaccinated women.

HPV16 and/or 18 infection

In the younger age-groups, HPV16/18 prevalence decreased within the post-vaccination period between 2010/11 to 2016 from 8.2% to 1.6% in 16-18 year olds and 14.0% to 1.6% in 19-21 year olds (compared to 17.6% and 16.9% in the pre-vaccination period[19] respectively; p-trend for both age-groups <0.001; Table 3, Figure 1). These decreases were strongly associated with the increasing estimated vaccination coverage (aPR (95% CI), 0.2 (0.1-0.3) and 0.3 (0.2-0.4) for 16-18 and 19-21 year olds respectively). In the oldest age-group, the prevalence in 2014-2015 was 7.5% compared to 16.4% 2010-2011 (15.3% in the pre-vaccination period[19]; p-trend 0.417) although when we considered changes relative to vaccination coverage, there was evidence of an association (aPR=0.3 (0.1-0.6)) (Table 3). Observed percentage reductions were similar to model predictions for both HPV types (Figure 3).

The prevalence of HPV16/18 in cohorts offered routine vaccination was 2.0% (1.5-2.4%) and 1.3% (0.7%-2.0%) in those aged 16-18 years and 19-21 years at sample collection respectively.

Among the subset of women with known vaccination status, full-course vaccine-effectiveness against HPV16/18 was 82.0 (60.6-91.8) for women vaccinated <15 years and 48.7 (20.8-66.8) for women vaccinated at 15-17 years (Table 4, Figure 2).

HPV31, 33 and/or 45 infection

In the younger age-groups, there was evidence of a decrease in the prevalence of HPV31/33/45 within the post vaccination period (6.5% to 0.6% for 16-18 year olds and 8.6% to 2.6% for 19-21 year olds; Table 3, Figure 1). These reductions were associated with estimated vaccination coverage (aPRs for HPV31/33/45 were 0.3 (0.2-0.5) and 0.5 (0.3-0.7) for 16-18 and 19-21 year olds respectively).

Restricting to women with a known vaccination status, there was also evidence of a protective effect of receiving the vaccine (vaccine-effectiveness against types HPV31/33/45 of 54.3 (8.6-77.2) for the women vaccinated <15 years and 36.7 (-3.4-61.2) for women vaccinated at age 15-17) (Table 4, Figure 2).

Non-vaccine HR HPV types

In 16-18 year olds, the prevalence of non-16/18/31/33/45 HPV types remained relatively stable at 31.0% in 2010-2011 to 26.7% in 2016 (p-trend 0.211). The prevalence also remained relatively stable for 19-21 year olds (34.0% in 2010-2011 to 32.9% in 2016; p=0.877) but there was a slight increase among 22-24 year olds (27.0% in 2010-2011 to 31.7% in 2014; p<0.001).

The prevalence of HPV6/11 remained relatively constant over time (Table 3). Among those with HPV vaccination status, there was no protective effect of the bivalent vaccine against these HPV types (vaccine-effectiveness 26.5 (-26.8-57.4)

for women vaccinated <15 years and 18.8 (-24.6-47.1) for women vaccinated at 15-17 years).

Sensitivity analysis

Restricting vaccine-effectiveness estimates to women who were known to have three HPV vaccine doses within the recommended schedule gave very similar results to the main analysis for HPV16/18 (vaccine-effectiveness (95% CI) of 81.7% (58.8-91.8%) for vaccination <15 years and 47.9% (17.7-67.1%) for vaccination at age 15-17).

Herd protection

In 16-21 year old women, the prevalence of HPV16 /18 in those unvaccinated (either with known vaccination status or not eligible to receive the HPV vaccine) reduced from 15.9% in 2010-2011 (n=141/885) to 12.5% in 2012-2013 (n=56/449) to 6.9% in 2014-2016 (n=5/72) (p-trend=0.013). For HPV31/33/45, the prevalence estimates were 9.4% (2010-2011), 10.7% (2012-2013) and 6.9% (2014-2016); p-trend=0.577.

Discussion

We have previously reported changes in the prevalence of HPV types between the pre- and the post-vaccination period[90, 126]. Interpretation of these findings was complicated by a change in the HPV assay used between the pre- and post-vaccination periods which affected the detection of some non-vaccine HPV types. In this report, we consider changes over time within the post-vaccination period during which testing was conducted consistently with our in-house assay. If HPV vaccination is causing a decrease or increase in certain HPV types we expect to see these decreases or increases strengthening over time since vaccination, as later periods include women vaccinated at a younger age and with higher vaccination coverage. Additionally, for a subset of women with vaccination status, we have

calculated vaccine-effectiveness, directly comparing HPV prevalence in vaccinated and unvaccinated women.

Among ages offered HPV vaccination, we have demonstrated clear reductions in infections with the HPV vaccine types since the introduction of the HPV vaccination programme in England, with greater reductions in younger women (with higher vaccination coverage and vaccinated at a younger age).Vaccine-effectiveness in those vaccinated <15 years was high, as expected given the high vaccine efficacy from per-protocol analyses of clinical trials[128, 129, 132, 133]. The lower effectiveness in the older catch-up females is also to be expected, given risk of prior exposure to HPV. We have also demonstrated that these declines are associated with increasing vaccination coverage within the post-vaccination period. We have compared percentage declines in HPV vaccine types in this surveillance with predicted outputs from a dynamic transmission model conducted prior to the introduction of HPV vaccination in England[137]. These results provide reassurance that observed declines in HPV16/18 prevalence are similar to what was expected.

Encouragingly, there is also evidence of a substantial herd protection effect with HPV16/18 prevalence also reducing over time in unvaccinated women which is consistent with data from other countries[95, 96, 135]. We have also seen clear evidence of cross-protection following introduction of a bivalent vaccine with declines in HPV31/33/45 within the post-vaccination period overall. In the younger age groups, percentage reductions over time have been similar for vaccine types and HPV31/33/45 (Figure 1) despite lower vaccine-effectiveness for HPV31/33/45 (54.3% vs 82.0% in those vaccinated age 15 or younger). This is consistent with predictions from mathematical modelling; that the lower basic reproductive number for some HPV types means herd protective effects could be greater [138] (i.e. it may be easier to reduce prevalence of types with a lower basic reproductive number

(such as HPV18, HPV31, HPV33 and HPV45) as herd protection effects are stronger).

We have previously reported increases in non-vaccine types between the pre- and post-vaccination periods and potential reasons for this were discussed[90]. In this analysis we have seen stable prevalence of non-16/18/31/33/45 HR types within the post-vaccination period. This is not consistent with what we would expect to see if vaccination against HPV16/18 was driving increases of other non-vaccine types, or type-replacement. This supports our caution in prior discussion of the changes between the pre-vaccination and the post-vaccination period to end 2014, and suggests these were likely unrelated to HPV vaccination.

Our results for reductions in the HPV vaccine types are consistent with elsewhere in the UK and worldwide. A systematic review and meta-analysis including data from Scotland, England, US and Australia demonstrated decreases in HPV16/18 prevalence among 13-19 year old women (risk ratio 0.32 (95% CI 0.19-0.52))[135]. A large study of over 12,000 samples from 13-22 year old women attending for chlamydia screening in Sweden showed a reduction in HPV16 (from 14.9% pre-vaccination to 8.7% post-vaccination) and HPV18 (7.9% to 4.3%)[91]. Another study of 1,087 16-22 year old women which was conducted in The Netherlands compared HPV prevalence in vaccinated vs. unvaccinated women and demonstrated a slightly higher vaccine-effectiveness against HPV16/18 of 89.9% (81.7-94.4%)[139]. Updated data from Scotland has reported similar results with a vaccine-effectiveness against HPV16/18 of 89% (85-92%) among those vaccinated at age 12-13 years old[95]. However, evidence for changes in the non-vaccine types is less consistent across different studies. The meta-analysis conducted by Drolet et al demonstrated a reduction in HPV31/33/45 combined (RR 0.72 (0.54-0.96)) for both vaccines combined. However, a related systematic review and meta-analysis which considered changes in individual non-vaccine types only demonstrated reductions in

HPV31 in women ≤19 years old but not HPV33 or HPV45[136]. In a study in Sweden, where the quadrivalent vaccine has been used, there was also some suggestion of a reduction in HPV31 but not in the other closely related HPV types[91]. However, a more recent study of 8,584 20-21 year old women attending for cervical screening in Scotland, where the bivalent vaccine was used, demonstrated a lower prevalence among vaccinated women compared to unvaccinated women for types HPV31/33/45 individually and a very high combined vaccine-effectiveness of 85.1% and 83.6% in women vaccinated at 12-13 years and 14 years respectively[95]. Similarly, data from The Netherlands who adopted the bivalent vaccine has also shown lower prevalence of HPV31/33/45 among vaccinated compared to unvaccinated women[139].

There are certain limitations of our surveillance which should be taken into account when interpreting these results. Firstly, analyses comparing changes in HPV prevalence over time may reflect changes due to HPV vaccination; however, other changes at a population level, or individual differences not adjusted for in our analysis, should not be ruled out. We have adjusted for the testing venue, age and chlamydia positivity, but the extent to which changes in sexual behaviour (in addition to the adjustment for chlamydia positivity) or other unrecorded changes have affected HPV prevalence estimates is unclear. Secondly, there may be inequalities in HPV vaccination uptake which affect our analyses of vaccine-effectiveness and estimation of herd protection effects. We have compared characteristics by vaccination status (Table 2) and, similarly to the above, we have attempted to address these inequalities by adjusting vaccine-effectiveness for venue type, age and chlamydia positivity. However, there may be other factors associated with vaccine uptake and HPV prevalence which have not been accounted for. For example, for both analyses we have missing data on ethnicity which is a potential confounder as it is known to be associated with HPV vaccination uptake and HPV

prevalence. Due to the large proportion of missing data, particularly in more recent years, we were not able to adjust our estimates for ethnicity which may have biased our results. Calculation of vaccine-effectiveness was only possible for women with known vaccination status (21% of women eligible to receive the vaccine). The women with vaccination data available may not be representative of all women included in this study as missing vaccination status occurred largely at an area-level (either laboratories not providing NHS number or local CHIS systems not being able to conduct linkage). Over 80% of these specimens came from one testing laboratory in Cornwall. Vaccination in Cornwall differed from the vast majority of other areas in England as the vaccine was largely delivered during these years in primary care (rather than schools). As such, if there is any reason that this method of vaccine delivery could affect vaccine-effectiveness then these results may not be representative of the rest of England. For example, vaccination at primary care is likely to lead to vaccine doses given outside of the optimum schedule as individuals will need to be followed up individually for 2nd and 3rd doses rather than having mop-up sessions at schools which is likely to underestimate vaccine-effectiveness. However, excluding women who received 3-doses of the vaccine outside of the recommended schedule had little effect on the HPV16/18 vaccine-effectiveness (from 82.0% to 81.7% for 16-18 year olds). A further limitation of our analysis of vaccine-effectiveness is the relatively small number of unvaccinated women, particularly at younger ages. Consequently, some confidence intervals for vaccine-effectiveness are wide.

This large surveillance includes HPV results from over 15,000 samples which have allowed us to consider a breadth of analyses to monitor changes in the prevalence of HPV infection in young women since the introduction of national HPV vaccination. A key strength of this analysis is the ability to monitor the population effects of vaccination among vaccinated and unvaccinated women prior to these women

entering cervical screening, therefore offering information to inform screening services. We clearly demonstrate dramatic declines in the prevalence of vaccine HPV types in this population, similar to predictions from effectiveness models. We also demonstrate clear evidence of declining prevalence of HPV31/33/45, most likely attributable to cross-protection. As cervical screening is changing to HPV testing as a primary screen our data can inform decisions regarding HPV testing strategies. These data should also be used to inform assessments of the additional benefit of introducing the nonavalent vaccine to the national vaccination programme.

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Table 1: Patient characteristics by year of surveillance

	Post-vaccination			
	2010-2011	2012-2013	2014-2015	2016
	n=4,044	n=7,253	n=2,737	n=1,425
Number of samples by laboratory				
North West (Aintree)	203 (5.0%)	1198 (16.5%)	0 (0.0%)	0 (0.0%)
Yorkshire and The Humber (Leeds)	683 (16.9%)	1478 (20.4%)	790 (28.9%)	0 (0.0%)
West Midlands (Stoke)	344 (8.5%)	224 (3.1%)	199 (7.3%)	233 (16.4%)
East of England (Norfolk and Norwich)	222 (5.5%)	123 (1.7%)	0 (0.0%)	0 (0.0%)
East of England (Cambridge)	345 (8.5%)	697 (9.6%)	27 (1%)	272 (19.1%)
South East (East Kent)	563 (13.9%)	1326 (18.3%)	1048 (38.3%)	673 (47.2%)
South East (Portsmouth)	215 (5.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
South West (Cornwall)	566 (14.0%)	2028 (28.0%)	673 (24.6%)	247 (17.3%)
London (UCL)	476 (11.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
London (Lewisham)	427 (10.6%)	179 (2.5%)	0 (0.0%)	0 (0.0%)
Age - years (data completeness)	(100%)	(100%)	(100%)	(100%)
16-18 years	1128 (27.9%)	2094 (28.9%)	1953 (71.4%)	629 (44.1%)
19-21 years	1704 (42.1%)	2892 (39.9%)	664 (24.3%)	796 (55.9%)
22-24 years	1212 (30.0%)	2267 (31.3%)	120 (4.4%)	0 (0.0%)
Ethnicity (data completeness)	(75%)	(36%)	(37%)	(44%)
White	2393 (78.8%)	2292 (89.0%)	917 (91.6%)	566 (89.6%)
Black	399 (13.1%)	168 (6.5%)	51 (5.1%)	34 (5.4%)
Asian	80 (2.6%)	47 (1.8%)	16 (1.6%)	16 (2.5%)
Other	164 (6.9%)	68 (3.0%)	17 (1.9%)	16 (2.8%)
Sample collection venue (data completeness)	(100%)	(100%)	(100%)	(100%)
General practice (GP)	1212 (30.0%)	3018 (41.6%)	1321 (48.3%)	1230 (86.3%)
Family planning (Community Sexual Health Services; CaSH)	2714 (67.1%)	4029 (55.5%)	1416 (51.7%)	195 (13.7%)
Youth clinic	118 (2.9%)	206 (2.8%)	0 (0%)	0 (0%)
chlamydia positivity (data completeness)	8.3% (100%)	6.2% (100%)	6.3% (100%)	6% (100%)
HPV vaccination cohort (data completeness)	(100%)	(100%)	(100%)	(100%)

Routine cohorts (offered vaccination 12-13 years old)	13 (0.3%)	659 (9.1%)	2261 (82.6%)	1365 (95.8%)
Younger catch-up cohorts (offered vaccination 14-16 years old)	604 (14.9%)	1598 (22.0%)	279 (10.2%)	60 (4.2%)
Older catch-up cohorts (offered vaccination 17-18 years old)	1425 (35.2%)	2807 (38.7%)	127 (4.6%)	0 (0%)
Not eligible for HPV vaccination	2002 (49.5%)	2189 (30.2%)	70 (2.6%)	0 (0%)
HPV vaccination status in eligible cohorts (data completeness)	(13%)	(25%)	(19%)	(21%)
Fully vaccinated	119 (44.4%)	913 (73.6%)	440 (85.4%)	255 (86.7%)
Partially vaccinated	17 (6.3%)	69 (5.6%)	25 (4.9%)	11 (3.7%)
Vaccinated (unknown number of doses)[a]	37 (13.8%)	26 (2.1%)	0 (0%)	0 (0%)
Unvaccinated	95 (35.4%)	233 (18.8%)	50 (9.7%)	28 (9.5%)
Estimated 3-dose coverage in those with unknown vaccination status[b]	53.6%	58.6%	81.9%	83.3%

[a] Vaccination status data from Lewisham did not include the number of doses given was unavailable
[b] Estimated using national HPV vaccination coverage for relevant birth cohorts; these women were assumed to be fully vaccinated in our main analysis

Table 2: Patient characteristics comparing vaccinated and unvaccinated women in the post-vaccination period

	Vaccinated (n=1,912)	Unvaccinated (n=406)	Total with known vaccination status (n=2,318)	Unknown status (n=8,880)
Age - years (data completeness)	(100%)	(100%)	(100%)	(100%)
16-18 years	990 (51.8%)	123 (30.3%)	1,113 (48.0%)	4,691 (52.8%)
19-21 years	845 (44.2%)	263 (64.8%)	1,108 (47.8%)	3,928 (44.2%)
22-24 years	77 (4.0%)	20 (4.9%)	97 (4.2%)	261 (2.9%)
Ethnicity (data completeness)	(12%)	(32%)	(15%)	(52%)
White	191 (86.8%)	72 (56.3%)	263 (75.6%)	4,051 (88.4%)
Black	25 (11.4%)	49 (38.3%)	74 (21.3%)	321 (7.0%)
Asian	<5[a]	<5[a]	<5[a]	82 (1.8%)
Other	<5[a]	<10[a]	<10[a]	126 (2.8%)
Sample collection venue (data completeness)	(100%)	(100%)	(100%)	(100%)
General practice	1,554 (81.3%)	255 (62.8%)	1,809 (78.0%)	3,165 (35.6%)
Family planning (Community Sexual Health Services; CaSH)	326 (17.1%)	138 (34.0%)	464 (20.0%)	5,525 (62.2%)
Youth clinic	32 (1.7%)	13 (3.2%)	45 (1.9%)	190 (2.1%)
chlamydia positivity (data completeness)	6.4% (100%)	8.4% (100%)	6.7% (100%)	7.7% (100%)

[a] small numbers masked in line with Public Health England data sharing policy

Table 3: Post-vaccination type-specific HPV prevalence by age-group among all women

HPV type	Post-vaccination prevalence (95% CI) 2010-2011	2012-2013	2014-2015[a]	2016	p-value for trend[c]	Prevalence ratio associated with estimated vaccination coverage[d] Unadjusted (95% CI)	Adjusted[b] (95%CI)
16-18 years old	**n=1,128**	**n=2,094**	**n=1,953**	**n=629**			
[Estimated HPV vaccination coverage]	[60%]	[77%]	[84%]	[84%]			
Any High-risk HPV	37.7 (34.8, 40.5)	35.9 (33.9, 38.0)	33.8 (31.7, 35.9)	28.1 (24.6, 31.7)	<0.001	0.7 (0.6, 0.8)	-
Other high-risk HPV (not 16/18/31/33/45)	31.0 (28.3, 33.7)	31.1 (29.1, 33.1)	31.4 (29.3, 33.4)	26.9 (23.4, 30.3)	0.211	0.8 (0.7, 1.0)	-
Vaccine HPV types							
HPV16 and/or HPV18	8.2 (6.6, 9.9)	3.2 (2.5, 4.0)	1.8 (1.2, 2.4)	1.6 (0.6, 2.6)	<0.001	0.1 (0.1, 0.2)	0.2 (0.1, 0.3)
HPV16	6.4 (5.0, 7.8)	2.4 (1.7, 3.0)	1.5 (0.9, 2.0)	1.4 (0.5, 2.4)	<0.001	0.1 (0.1, 0.2)	0.2 (0.1, 0.3)
HPV18	2.8 (1.9, 3.8)	1.0 (0.5, 1.4)	0.4 (0.1, 0.7)	0.3 (-0.1, 0.8)	<0.001	0.1 (0.1, 0.3)	0.1 (0.1, 0.3)
Nonavalent HPV types							
HPV31/HPV33/HPV45/HPV52/HPV58	16.9 (14.7, 19.1)	14.7 (13.1, 16.2)	10.2 (8.8, 11.5)	7.2 (5.1, 9.2)	<0.001	0.4 (0.3, 0.6)	0.6 (0.4, 0.8)
HPV31/HPV33/HPV45	6.5 (5.0, 7.9)	5.8 (4.8, 6.8)	2.8 (2.1, 3.6)	0.6 (0.0, 1.3)	<0.001	0.2 (0.2, 0.4)	0.3 (0.2, 0.5)
HPV31	0.9 (0.3, 1.4)	1.7 (1.1, 2.2)	0.3 (0.0, 0.5)	0.2 (-0.2, 0.5)	0.001	0.1 (0.1, 0.3)	0.2 (0.1, 0.4)
HPV33	3.1 (2.1, 4.1)	2.6 (1.9, 3.3)	1.8 (1.2, 2.4)	0.3 (-0.1, 0.8)	<0.001	0.3 (0.1, 0.5)	0.3 (0.1, 0.6)
HPV45	2.7 (1.8, 3.7)	1.7 (1.2, 2.3)	0.8 (0.4, 1.2)	0.2 (-0.2, 0.5)	<0.001	0.3 (0.1, 0.7)	0.3 (0.1, 0.9)
HPV52	8.2 (6.6, 9.9)	6.5 (5.5, 7.6)	6.6 (5.5, 7.7)	4.9 (3.2, 6.6)	0.015	0.7 (0.5, 1.2)	1.1 (0.7, 1.9)
HPV58	3.8 (2.7, 4.9)	3.8 (3.0, 4.6)	1.7 (1.1, 2.3)	2.1 (1.0, 3.2)	<0.001	0.4 (0.2, 0.7)	0.6 (0.3, 1.2)
HPV6/11	7.8 (6.2, 9.4)	9.5 (8.2, 10.8)	10.7 (9.3, 12.1)	8.3 (6.1, 10.4)	0.181	0.7 (0.5, 1.0)	0.8 (0.5, 1.2)
HPV6	4.7 (3.5, 5.9)	5.5 (4.5, 6.5)	6.2 (5.2, 7.3)	4.0 (2.4, 5.5)	0.650	0.9 (0.5, 1.5)	1.1 (0.6, 1.9)
HPV11	4.4 (3.2, 5.6)	5.4 (4.5, 6.4)	6.3 (5.2, 7.4)	5.4 (3.6, 7.2)	0.136	0.6 (0.3, 0.9)	0.6 (0.4, 1.0)
19-21 years old	**n=1,704**	**n=2,892**	**n=664**	**n=796**			
[Estimated HPV vaccination coverage]	[25%]	[49%]	[79%]	[84%]			
Any High-risk HPV	45.8 (43.5, 48.2)	46.4 (44.6, 48.3)	39.5 (35.7, 43.2)	35.3 (32.0, 38.6)	<0.001	0.8 (0.8, 0.9)	0.9 (0.8, 1)
Other high-risk HPV (not 16/18/31/33/45)	34.0 (31.8, 36.3)	38.9 (37.2, 40.7)	35.8 (32.2, 39.5)	32.9 (29.6, 36.2)	0.877	1.0 (0.9, 1.1)	1.1 (1.0, 1.2)
Vaccine HPV types							
HPV16 and/or HPV18	14.0 (12.4, 15.7)	8.1 (7.1, 9.0)	2.7 (1.5, 3.9)	1.6 (0.8, 2.5)	<0.001	0.2 (0.2, 0.3)	0.3 (0.2, 0.4)
HPV16	11.0 (9.5, 12.5)	6.7 (5.8, 7.6)	1.8 (0.8, 2.8)	1.5 (0.7, 2.4)	<0.001	0.3 (0.2, 0.4)	0.3 (0.2, 0.5)
HPV18	3.6 (2.7, 4.5)	1.8 (1.3, 2.2)	0.9 (0.2, 1.6)	0.1 (-0.1, 0.4)	<0.001	0.1 (0.1, 0.2)	0.1 (0.1, 0.3)

Nonavalent HPV types							
HPV31/HPV33/HPV45/HPV52/HPV58	21.6 (19.6, 23.6)	21.0 (19.5, 22.5)	16.1 (13.3, 18.9)	12.7 (10.4, 15)	<0.001	0.7 (0.6, 0.8)	0.7 (0.6, 0.9)
HPV31/HPV33/HPV45	8.6 (7.3, 10.0)	8.2 (7.2, 9.2)	4.2 (2.7, 5.7)	2.6 (1.5, 3.8)	<0.001	0.4 (0.3, 0.5)	0.5 (0.3, 0.7)
HPV31	2.3 (1.6, 3.1)	2.7 (2.1, 3.2)	0.8 (0.1, 1.4)	0.6 (0.1, 1.2)	<0.001	0.3 (0.2, 0.6)	0.5 (0.3, 0.9)
HPV33	2.9 (2.1, 3.7)	3.3 (2.6, 3.9)	2.3 (1.1, 3.4)	1.3 (0.5, 2.0)	0.003	0.5 (0.3, 0.8)	0.6 (0.3, 1.0)
HPV45	3.6 (2.7, 4.5)	2.6 (2.0, 3.2)	1.4 (0.5, 2.2)	0.9 (0.2, 1.5)	<0.001	0.3 (0.2, 0.5)	0.4 (0.2, 0.6)
HPV52	10.3 (8.8, 11.7)	10.9 (9.7, 12.0)	9.2 (7.0, 11.4)	7.4 (5.6, 9.2)	0.020	0.8 (0.7, 1.0)	0.9 (0.7, 1.1)
HPV58	4.9 (3.8, 5.9)	4.9 (4.1, 5.7)	5.1 (3.4, 6.8)	3.8 (2.4, 5.1)	0.352	0.9 (0.6, 1.2)	0.8 (0.5, 1.2)
HPV6/11	8 (6.7, 9.3)	9.0 (7.9, 10.0)	9.2 (7.0, 11.4)	7.0 (5.3, 8.8)	0.752	1.1 (0.8, 1.4)	1.1 (0.8, 1.4)
HPV6	5.2 (4.1, 6.2)	4.9 (4.2, 5.7)	5.9 (4.1, 7.7)	3.1 (1.9, 4.4)	0.451	0.9 (0.7, 1.3)	0.9 (0.6, 1.4)
HPV11	3.9 (3.0, 4.9)	5.4 (4.6, 6.3)	5.4 (3.7, 7.1)	4.8 (3.3, 6.3)	0.135	1.3 (0.9, 1.7)	1.2 (0.8, 1.8)
22-24 years old	**n=1,212**	**n=2,267**	**n=120**	**n=0**			
[Estimated HPV vaccination coverage]	[0%]	[7%]	[25%]				
Any High-risk HPV	40.3 (37.5, 43.0)	46.8 (44.8, 48.9)	40.0 (31.1, 48.9)		<0.001	1.0 (0.8, 1.2)	1.0 (0.8, 1.3)
Other high-risk HPV (not 16/18/31/33/45)	27.0 (30.8, 34.6)	32.7 (30.8, 34.6)	31.7 (23.2, 40.1)		<0.001	1.4 (1.1, 1.7)	1.4 (1.1, 1.8)
Vaccine HPV types							
HPV16 and/or HPV18	16.4 (14.3, 18.5)	15.9 (14.4, 17.4)	7.5 (2.7, 12.3)		0.417	0.3 (0.2, 0.6)	0.3 (0.1, 0.6)
HPV16	14.6 (12.6, 16.6)	13.4 (12, 14.8)	5.8 (1.6, 10.1)		0.956	0.3 (0.2, 0.6)	0.3 (0.1, 0.6)
HPV18	2.6 (1.7, 3.5)	3.1 (2.4, 3.8)	1.7 (-0.7, 4.0)		0.008	0.3 (0.1, 1.5)	0.3 (0.1, 1.3)
Nonavalent HPV types							
HPV31/HPV33/HPV45/HPV52/HPV58	18.4 (16.2, 20.6)	23.3 (21.6, 25.1)	18.3 (11.3, 25.4)		0.001	1.1 (0.8, 1.5)	1.2 (0.8, 1.7)
HPV31/HPV33/HPV45	7.8 (6.3, 9.4)	10.9 (9.7, 12.2)	8.3 (3.3, 13.4)		0.016	0.6 (0.3, 1.2)	0.6 (0.3, 1.3)
HPV31	2.5 (1.6, 3.4)	3.1 (2.4, 3.8)	2.5 (-0.3, 5.3)		0.501	0.7 (0.2, 2.2)	0.5 (0.2, 2.0)
HPV33	2.1 (1.3, 2.9)	3.3 (2.6, 4.0)	3.3 (0.1, 6.6)		0.155	0.9 (0.3, 2.6)	0.8 (0.2, 2.4)
HPV45	3.6 (2.6, 4.7)	4.8 (3.9, 5.7)	2.5 (-0.3, 5.3)		0.096	0.5 (0.2, 1.4)	0.6 (0.2, 1.9)
HPV52	8.6 (7.0, 10.2)	11.0 (9.7, 12.3)	7.5 (2.7, 12.3)		0.010	1.5 (1.0, 2.4)	1.6 (1.0, 2.7)
HPV58	3.2 (2.2, 4.2)	3.7 (2.9, 4.5)	5.0 (1.0, 9.0)		0.261	1.9 (0.9, 4.1)	2.0 (0.9, 4.6)
HPV6/11	3.5 (2.4, 4.5)	6.0 (5.0, 6.9)	1.7 (-0.7, 4.0)		0.008	1.9 (1.0, 3.5)	1.5 (0.8, 3.1)
HPV6	1.3 (0.7, 2.0)	3 (2.3, 3.7)	0.8 (-0.8, 2.5)		<0.001	1.7 (0.6, 4.5)	1.1 (0.4, 3.2)
HPV11	2.1 (1.3, 3.0)	3.6 (2.8, 4.4)	0.8 (-0.8, 2.5)		0.288	2.0 (0.9, 4.4)	1.9 (0.8, 4.5)

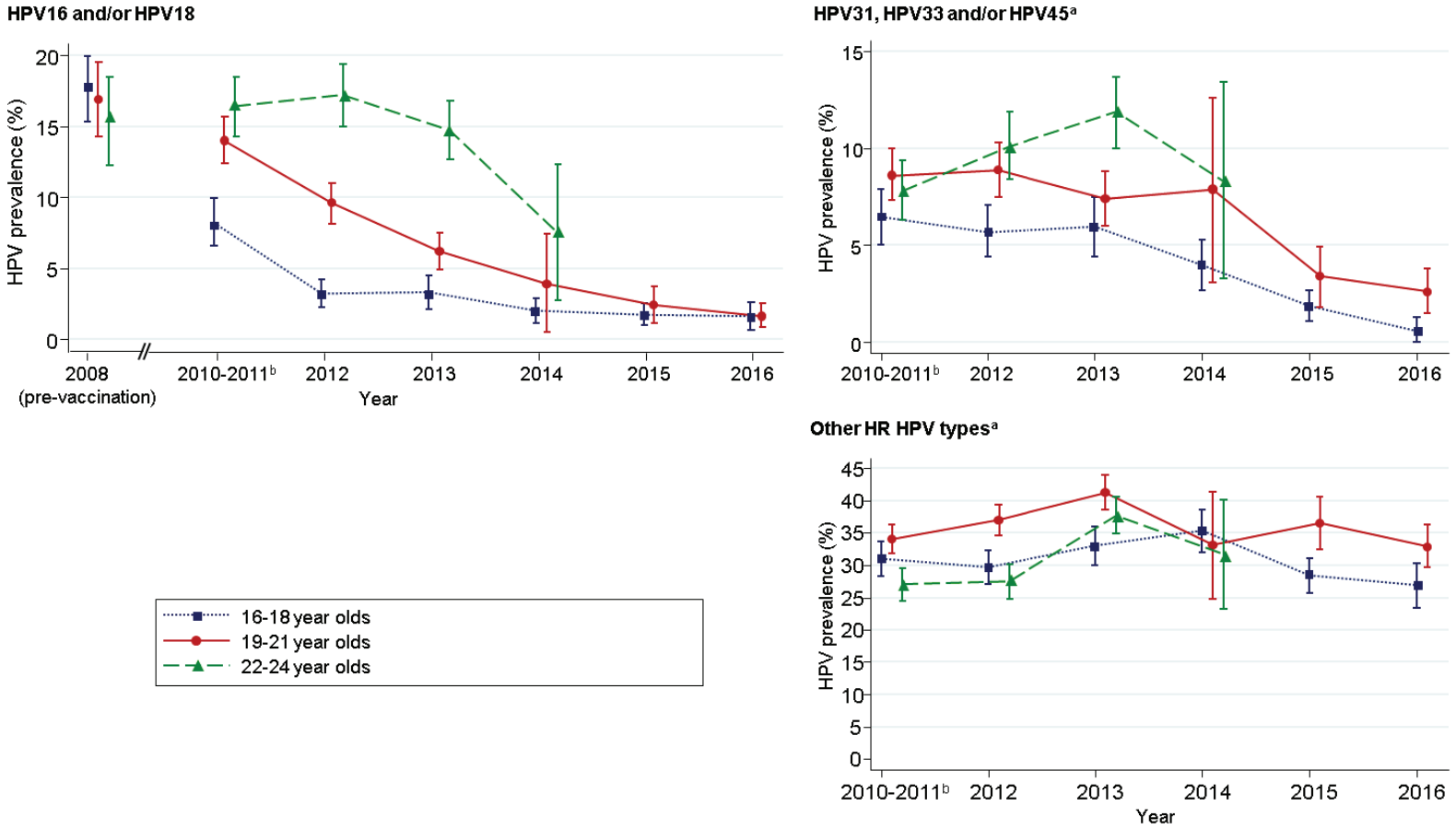
[a] No samples were collected for 22-24 year olds in 2015 or 2016; [b] Adjusted for age, venue type and chlamydia positivity; [c] p-values for trend compare changes in prevalence over time using a log-binomial regression model, adjusted for age, testing venue and chlamydia positivity; [d] Prevalence ratios for the association between estimated HPV vaccination coverage and changes in post-vaccination HPV prevalence

Table 4: Vaccine-effectiveness for the bivalent vaccine comparing HPV infection in the post-vaccination period, by age group (2010-2016)

	Unvaccinated	Fully vaccinated[a]	Fully vaccinated[b] (95% CI)
Younger HPV vaccination cohorts[c] combined	**n=117**	**n=1,176**	
HPV 16/18 with or without other HR types	10 (8.5%)	16 (1.4%)	82.0 (60.6, 91.8)
Non-vaccine type(s) with or without HPV 16/18			
HPV 31/33/45	9 (7.7%)	42 (3.6%)	54.3 (8.6, 77.2)
HPV 31/33/45/52/58	18 (15.4%)	138 (11.7%)	16.4 (-30.9, 46.5)
HPV6/11	13 (11.1%)	98 (8.3%)	26.5 (-26.8, 57.4)
Older catch-up HPV vaccination cohorts	**n=289**	**n=614**	
HPV 16/18 with or without other HR types	39 (13.5%)	38 (6.2%)	48.7 (20.8, 66.8)
Non-vaccine type(s) with or without HPV 16/18			
HPV 31/33/45	28 (9.7%)	35 (5.7%)	36.7 (-3.4, 61.2)
HPV 31/33/45/52/58	70 (24.2%)	116 (18.9%)	20.6 (-3.5, 39.1)
HPV6/11	31 (10.7%)	52 (8.5%)	18.8 (-24.6, 47.1)

[a] Excludes 122 women who were partially vaccinated
[b] Adjusted for age, venue type and chlamydia positivity
[c] Including routine cohorts and younger vaccination cohorts (i.e. all women offered vaccination at age 15 or younger)

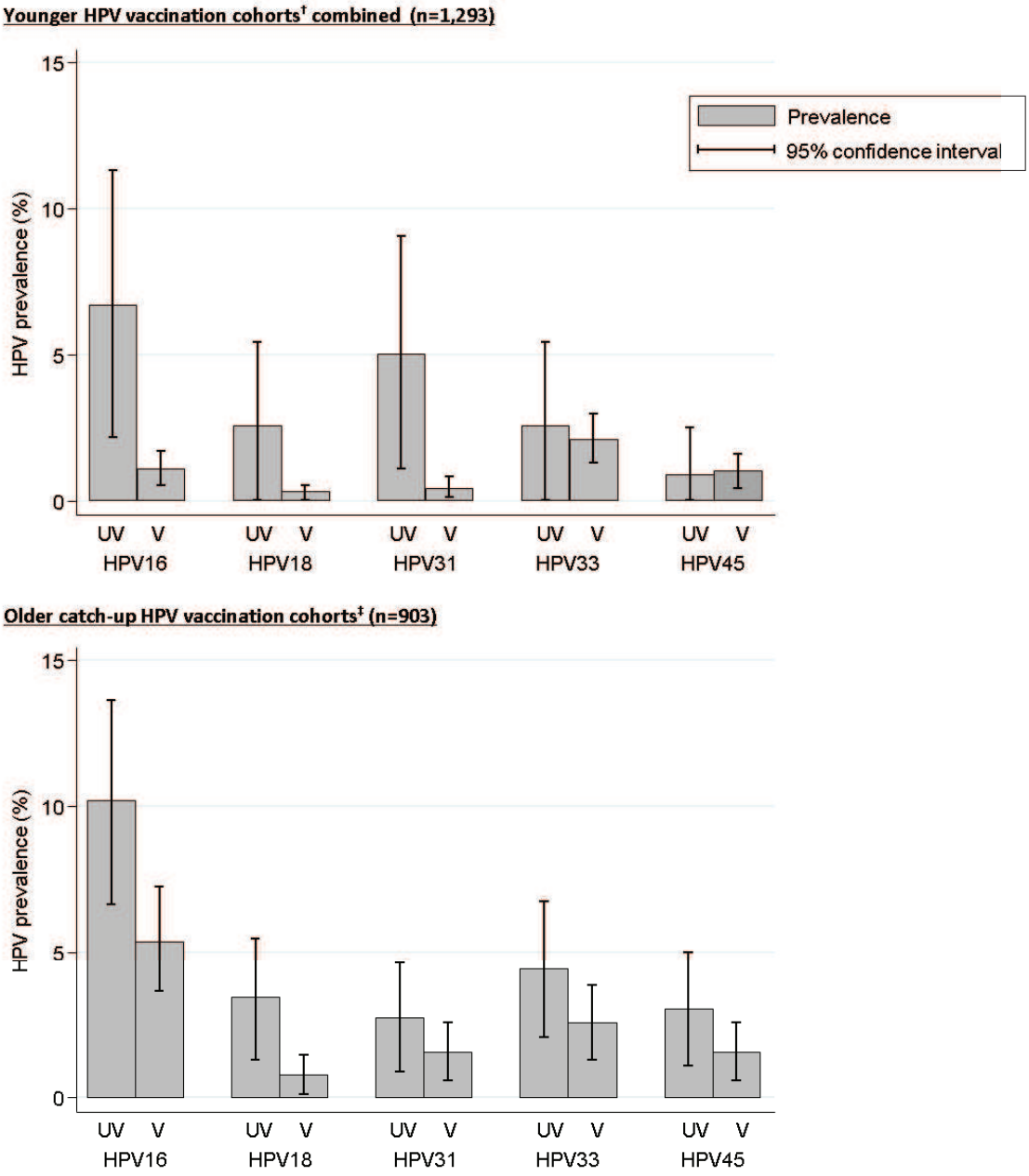
Figure 1: Prevalence of HPV infection by year of sample collection



a Pre-vaccination prevalence not given for non-vaccine HPV types due to the assay change between the pre- and post-vaccination period

b Data for 2010 and 2011 were combined as very few samples were collected in 2010 and most laboratories started collection from 2011

Figure 2: Prevalence of HPV infection by year of sample collection

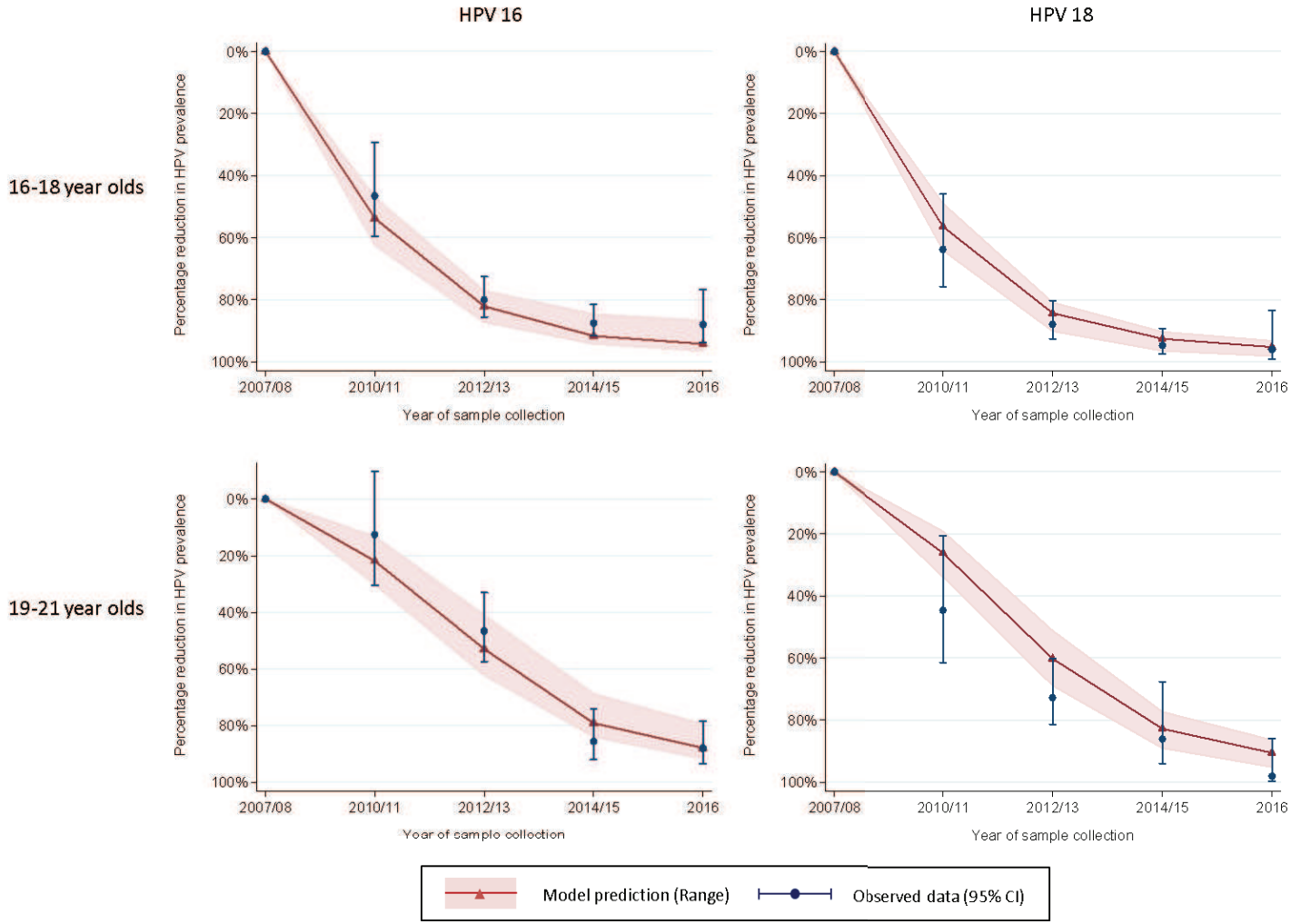


UV = Unvaccinated; V = Fully vaccinated

† Younger HPV vaccination cohorts includes females offered routine vaccination (age 12 years old) and younger catch-up vaccination cohorts (i.e. females offered vaccination at age 15 or younger)

‡ Older catch-up vaccination cohorts includes females offered vaccination at age 16, 17 and 18 years old

Figure 3: Observed percentage reductions in HPV16 and HPV18 prevalence compared to previous model predictions



6.4. Further discussion of potential bias in the results of HPV infection surveillance

6.4.1. The effect of the assay change between the pre-vaccination and post-vaccination period

The paper in Section 6.2 compared the prevalence of HPV between the pre-vaccination and post-vaccination period. As described in Section 6.3, the interpretation of these results was inhibited by the change in assay between the two time periods. Therefore, in the updated analyses presented in Section 6.3, I investigated changes within the post-vaccination period (during which time the assay did not change), and the association of these changes with increased vaccination coverage over time. However, for completeness, I include in this Section the updated results comparing the HPV prevalence in the pre-vaccination period with the post-vaccination period after applying the methods that I developed in this thesis to adjust for the effect of the different assay between the two time periods.

In Section 4.4.3, I describe methods which I used to adjust the pre-vaccination prevalence for the assay change in the post-vaccination period. This adjusted prevalence represents the prevalence that would be expected if these samples were tested using the post-vaccination assay. This adjustment made use of results from a validation study which compared the two assays and was conducted outside of this PhD (the results are shown in Appendix C1). Table 6.1 shows the unadjusted and adjusted pre-vaccination prevalence for the HPV infection surveillance alongside the post-vaccination HPV prevalence for comparison. For all age-groups, the pre-vaccination prevalence was similar with and without adjustment for HPV16/18 combined and HPV31/33/45/52/58 combined although the adjusted prevalence was slightly lower for type-specific results for HPV18, HPV31 and HPV58. Conversely, the adjusted pre-vaccination prevalence for the non-vaccine high-risk HPV types combined was higher than the unadjusted pre-vaccination prevalence.

In Section 4.4.4, I described the methods I developed to adjust estimates of the odds ratio for type-specific HPV in the pre-vaccination period compared with the post-vaccination period. Table 6.2 shows the unadjusted odds ratio and prevalence ratio, the odds ratio and prevalence ratio adjusted for known demographic and behaviour characteristics and the odds ratio additionally adjusted for the change in the assay between the two periods. Similar to the previous results (presented in the first paper in this Chapter; Section 6.2), the unadjusted and adjusted prevalence ratios and odds ratios were similar for HPV16/18 combined and demonstrated a clear reduction in these types following the introduction of HPV vaccination. There was also some evidence of a protective effect of HPV vaccination against HPV31/33/45 infection although, after adjustment for the assay change, this was less clear due to the additional uncertainty which widened the confidence intervals for the odds ratios. For all age-groups, the odds ratios and prevalence ratios for other high-risk HPV types combined were greater than 1.0, suggesting that the prevalence of these HPV types increased following vaccine introduction. In line with the results in Table 6.1, the odds ratio reduced (towards the null) after adjustment for the assay change although there was still some evidence of increases, particularly in the older age-groups. I explore possible reasons for this increase in detail in the discussion of the first paper in this Chapter (Section 6.2) and further in Section 6.5.1 below.

Table 6.1: Pre- and post-vaccination HPV prevalence in the HPV infection surveillance; with adjustment of pre-vaccination prevalence for the assay change between the two periods (including specimens collected to the end-December 2016)

HPV type	Pre-vaccination prevalence (95% CI)	Adjusted pre-vaccination prevalence (95% CI)[1]	Post-vaccination prevalence (95% CI)			
			2010-2011	2012-2013	2014-2015	2016
16-18 years old	**n=1,047**	**n=1,047**	**n=1,128**	**n=2,094**	**n=1,953**	**n=629**
[Estimated HPV vaccination coverage]	[0%]	[0%]	[60%]	[77%]	[84%]	[84%]
Any High-risk HPV	32.6 (29.7, 35.4)	34.8 (27.7, 41.6)	37.7 (34.8, 40.5)	35.9 (33.9, 38.0)	33.8 (31.7, 35.9)	28.1 (24.6, 31.7)
Any other high-risk HPV	24.9 (22.3, 27.6)	29.6 (23.9, 35.9)	31.0 (28.3, 33.7)	31.1 (29.1, 33.1)	31.4 (29.3, 33.4)	26.9 (23.4, 30.3)
Vaccine HPV types						
HPV16 and/or HPV18	17.6 (15.3, 19.9)	18.0 (13.5, 22.7)	8.2 (6.6, 9.9)	3.2 (2.5, 4.0)	1.8 (1.2, 2.4)	1.6 (0.6, 2.6)
HPV16	11.9 (10.0, 13.9)	12.8 (9.2, 16.8)	6.4 (5.0, 7.8)	2.4 (1.7, 3.0)	1.5 (0.9, 2.0)	1.4 (0.5, 2.4)
HPV18	7.8 (6.2, 9.5)	6.1 (3.1, 9.5)	2.8 (1.9, 3.8)	1.0 (0.5, 1.4)	0.4 (0.1, 0.7)	0.3 (-0.1, 0.8)
Nonavalent HPV types						
HPV31/HPV33/HPV45/HPV52/HPV58	14.5 (12.4, 16.7)	14.6 (10.4, 18.9)	16.9 (14.7, 19.1)	14.7 (13.1, 16.2)	10.2 (8.8, 11.5)	7.2 (5.1, 9.2)
HPV31/HPV33/HPV45	8.4 (6.7, 10.1)	7.2 (3.8, 10.9)	6.5 (5.0, 7.9)	5.8 (4.8, 6.8)	2.8 (2.1, 3.6)	0.6 (0.0, 1.3)
HPV31	3.7 (2.6, 4.9)	2.3 (0.2, 4.6)	0.9 (0.3, 1.4)	1.7 (1.1, 2.2)	0.3 (0.0, 0.5)	0.2 (-0.2, 0.5)
HPV33	2.4 (1.5, 3.3)	2.3 (0.4, 4.6)	3.1 (2.1, 4.1)	2.6 (1.9, 3.3)	1.8 (1.2, 2.4)	0.3 (-0.1, 0.8)
HPV45	2.9 (1.9, 3.9)	3.3 (1.0, 6.1)	2.7 (1.8, 3.7)	1.7 (1.2, 2.3)	0.8 (0.4, 1.2)	0.2 (-0.2, 0.5)
HPV52	4.0 (2.8, 5.2)	4.7 (2.2, 7.3)	8.2 (6.6, 9.9)	6.5 (5.5, 7.6)	6.6 (5.5, 7.7)	4.9 (3.2, 6.6)
HPV58	3.7 (2.6, 4.9)	2.8 (1.2, 4.7)	3.8 (2.7, 4.9)	3.8 (3.0, 4.6)	1.7 (1.1, 2.3)	2.1 (1.0, 3.2)
HPV6/11	5.8 (4.4, 7.2)	5.8 (3.1, 9.1)	7.8 (6.2, 9.4)	9.5 (8.2, 10.8)	10.7 (9.3, 12.1)	8.3 (6.1, 10.4)
HPV6	4.8 (3.5, 6.1)	3.6 (1.3, 6.2)	4.7 (3.5, 5.9)	5.5 (4.5, 6.5)	6.2 (5.2, 7.3)	4.0 (2.4, 5.5)
HPV11	1.4 (0.7, 2.2)	6.7 (0.0, 12.0)	4.4 (3.2, 5.6)	5.4 (4.5, 6.4)	6.3 (5.2, 7.4)	5.4 (3.6, 7.2)
19-21 years old	**n=804**	**n=804**	**n=1,704**	**n=2,892**	**n=664**	**n=796**
[Estimated HPV vaccination coverage]	[0%]	[0%]	[25%]	[49%]	[79%]	[84%]
Any High-risk HPV	34.3 (31.0, 37.6)	37.0 (29.6, 44.3)	45.8 (43.5, 48.2)	46.4 (44.6, 48.3)	39.5 (35.7, 43.2)	35.3 (32.0, 38.6)
Any other high-risk HPV	26.9 (23.8, 29.9)	32.3 (26.1, 39.2)	34.0 (31.8, 36.3)	38.9 (37.2, 40.7)	35.8 (32.2, 39.5)	32.9 (29.6, 36.2)
Vaccine HPV types						
HPV16 and/or HPV18	16.9 (14.3, 19.5)	17.1 (12.5, 22.1)	14.0 (12.4, 15.7)	8.1 (7.1, 9.0)	2.7 (1.5, 3.9)	1.6 (0.8, 2.5)
HPV16	12.6 (10.3, 14.9)	13.6 (9.7, 18.0)	11.0 (9.5, 12.5)	6.7 (5.8, 7.6)	1.8 (0.8, 2.8)	1.5 (0.7, 2.4)
HPV18	6.5 (4.8, 8.2)	4.5 (1.5, 7.8)	3.6 (2.7, 4.5)	1.8 (1.3, 2.2)	0.9 (0.2, 1.6)	0.1 (-0.1, 0.4)

Nonavalent HPV types						
HPV31/HPV33/HPV45/HPV52/HPV58	15.2 (12.7, 17.7)	15.4 (11.0, 20.1)	21.6 (19.6, 23.6)	21.0 (19.5, 22.5)	16.1 (13.3, 18.9)	12.7 (10.4, 15)
HPV31/HPV33/HPV45	8.3 (6.4, 10.2)	7.1 (3.6, 11.1)	8.6 (7.3, 10.0)	8.2 (7.2, 9.2)	4.2 (2.7, 5.7)	2.6 (1.5, 3.8)
HPV31	4.7 (3.3, 6.2)	3.5 (1.2, 6.3)	2.3 (1.6, 3.1)	2.7 (2.1, 3.2)	0.8 (0.1, 1.4)	0.6 (0.1, 1.2)
HPV33	2.0 (1.0, 3.0)	1.7 (-0.1, 4)	2.9 (2.1, 3.7)	3.3 (2.6, 3.9)	2.3 (1.1, 3.4)	1.3 (0.5, 2.0)
HPV45	2.6 (1.5, 3.7)	2.9 (0.6, 5.8)	3.6 (2.7, 4.5)	2.6 (2.0, 3.2)	1.4 (0.5, 2.2)	0.9 (0.2, 1.5)
HPV52	4.1 (2.7, 5.5)	4.8 (2.2, 7.8)	10.3 (8.8, 11.7)	10.9 (9.7, 12.0)	9.2 (7.0, 11.4)	7.4 (5.6, 9.2)
HPV58	5.0 (3.5, 6.5)	4 (2.3, 6.5)	4.9 (3.8, 5.9)	4.9 (4.1, 5.7)	5.1 (3.4, 6.8)	3.8 (2.4, 5.1)
HPV6/11	5.8 (4.2, 7.5)	5.9 (2.9, 9.4)	8 (6.7, 9.3)	9.0 (7.9, 10.0)	9.2 (7.0, 11.4)	7.0 (5.3, 8.8)
HPV6	5.3 (3.8, 6.9)	4.3 (1.8, 7.3)	5.2 (4.1, 6.2)	4.9 (4.2, 5.7)	5.9 (4.1, 7.7)	3.1 (1.9, 4.4)
HPV11	0.5 (0.0, 1.0)	2.3 (-1.3, 5.7)	3.9 (3.0, 4.9)	5.4 (4.6, 6.3)	5.4 (3.7, 7.1)	4.8 (3.3, 6.3)
22-24 years old	**n=503**	**n=503**	**n=1,212**	**n=2,267**	**n=120**	**n=0**
[Estimated HPV vaccination coverage]	[0%]	[0%]	[0%]	[7%]	[25%]	
Any High-risk HPV	32.8 (28.7, 36.9)	35.1 (27.1, 43.0)	40.3 (37.5, 43.0)	46.8 (44.8, 48.9)	40.0 (31.1, 48.9)	
Any other high-risk HPV	26.4 (22.6, 30.3)	31.7 (24.8, 39.5)	27.0 (30.8, 34.6)	32.7 (30.8, 34.6)	31.7 (23.2, 40.1)	
Vaccine HPV types						
HPV16 and/or HPV18	15.3 (12.2, 18.5)	15.1 (10.1, 20.6)	16.4 (14.3, 18.5)	15.9 (14.4, 17.4)	7.5 (2.7, 12.3)	
HPV16	10.9 (8.2, 13.7)	11.5 (7.4, 16.3)	14.6 (12.6, 16.6)	13.4 (12, 14.8)	5.8 (1.6, 10.1)	
HPV18	5.8 (3.7, 7.8)	3.6 (0.4, 7.3)	2.6 (1.7, 3.5)	3.1 (2.4, 3.8)	1.7 (-0.7, 4.0)	
Nonavalent HPV types						
HPV31/HPV33/HPV45/HPV52/HPV58	16.7 (13.4, 20.0)	17.3 (12.2, 23.0)	18.4 (16.2, 20.6)	23.3 (21.6, 25.1)	18.3 (11.3, 25.4)	
HPV31/HPV33/HPV45	8.9 (6.4, 11.4)	7.9 (3.9, 12.7)	7.8 (6.3, 9.4)	10.9 (9.7, 12.2)	8.3 (3.3, 13.4)	
HPV31	3.2 (1.6, 4.7)	1.7 (-0.6, 4.5)	2.5 (1.6, 3.4)	3.1 (2.4, 3.8)	2.5 (-0.3, 5.3)	
HPV33	2.6 (1.2, 4.0)	2.6 (0.4, 5.8)	2.1 (1.3, 2.9)	3.3 (2.6, 4.0)	3.3 (0.1, 6.6)	
HPV45	4.2 (2.4, 5.9)	5.4 (2.3, 9.9)	3.6 (2.6, 4.7)	4.8 (3.9, 5.7)	2.5 (-0.3, 5.3)	
HPV52	5.2 (3.2, 7.1)	6.4 (3.2, 10.4)	8.6 (7.0, 10.2)	11.0 (9.7, 12.3)	7.5 (2.7, 12.3)	
HPV58	3.0 (1.5, 4.5)	2.0 (0.3, 4.4)	3.2 (2.2, 4.2)	3.7 (2.9, 4.5)	5.0 (1.0, 9.0)	
HPV6/11	4.4 (2.6, 6.2)	3.9 (0.9, 7.5)	3.5 (2.4, 4.5)	6.0 (5.0, 6.9)	1.7 (-0.7, 4.0)	
HPV6	3.8 (2.1, 5.4)	2.4 (-0.1, 5.5)	1.3 (0.7, 2.0)	3 (2.3, 3.7)	0.8 (-0.8, 2.5)	
HPV11	0.8 (0.0, 1.6)	3.7 (-1.3, 9.4)	2.1 (1.3, 3.0)	3.6 (2.8, 4.4)	0.8 (-0.8, 2.5)	

1: *The pre-vaccination prevalence was adjusted to provide an estimate of the prevalence if the specimens had been tested using the same assay as the post-vaccination specimens (see description in Section 4.4.3)*

Table 6.2: Unadjusted and adjusted prevalence ratio and odds ratio of HPV infection in the post-vaccination period compared to the pre-vaccination period, by age-group (including specimens collected to end-December 2016)

	Unadjusted		Adjusted for patient characteristics[1]		Adjusted for patient characteristics and assay change[2]
HPV type	Odds ratio (95% CI)	Prevalence ratio (95% CI)	Odds ratio (95% CI)	Prevalence ratio (95% CI)	Odds ratio (95% CI)
16-18 years old					
Any High-risk HPV	1.1 (1.0, 1.3)	1.1 (1.0, 1.2)	1.3 (1.1, 1.5)	- [3]	1.2 (0.9, 1.5)
Any other high-risk HPV	1.5 (1.3, 1.7)	1.3 (1.2, 1.5)	1.7 (1.5, 2.0)	- [3]	1.4 (1.0, 1.8)
Vaccine HPV types					
HPV16 and/or HPV18	0.2 (0.1, 0.2)	0.2 (0.2, 0.2)	0.2 (0.2, 0.2)	0.2 (0.2, 0.3)	0.2 (0.1, 0.3)
HPV16	0.2 (0.2, 0.3)	0.2 (0.2, 0.3)	0.2 (0.2, 0.3)	0.3 (0.2, 0.3)	0.2 (0.1, 0.3)
HPV18	0.1 (0.1, 0.2)	0.1 (0.1, 0.2)	0.2 (0.1, 0.2)	0.2 (0.1, 0.2)	0.2 (0.1, 0.5)
Nonavalent HPV types					
HPV31/HPV33/HPV45/HPV52/HPV58	0.9 (0.7, 1.0)	0.9 (0.7, 1.0)	1.0 (0.8, 1.2)	1.0 (0.9, 1.2)	1.0 (0.8, 1.5)
HPV31/HPV33/HPV45	0.5 (0.4, 0.6)	0.5 (0.4, 0.7)	0.6 (0.4, 0.7)	0.6 (0.5, 0.8)	0.7 (0.4, 1.3)
HPV31	0.2 (0.2, 0.3)	0.2 (0.2, 0.4)	0.3 (0.2, 0.4)	0.3 (0.2, 0.4)	0.5 (0.2, 3.2)
HPV33	0.9 (0.6, 1.4)	0.9 (0.6, 1.4)	1.0 (0.6, 1.6)	1.0 (0.6, 1.6)	1.1 (0.5, 3.7)
HPV45	0.5 (0.3, 0.8)	0.5 (0.3, 0.8)	0.6 (0.4, 0.9)	0.6 (0.4, 0.9)	0.5 (0.2, 1.3)
HPV52	1.7 (1.2, 2.4)	1.7 (1.2, 2.3)	2.0 (1.4, 2.8)	1.9 (1.4, 2.6)	1.7 (1.0, 3.2)
HPV58	0.8 (0.5, 1.1)	0.8 (0.6, 1.1)	0.9 (0.6, 1.4)	0.9 (0.6, 1.3)	1.3 (0.8, 3.1)
HPV6 and/or HPV11	1.7 (1.3, 2.2)	1.6 (1.3, 2.1)	1.9 (1.4, 2.5)	1.8 (1.4, 2.4)	1.9 (1.2, 3.4)
HPV6	1.1 (0.8, 1.6)	1.1 (0.8, 1.5)	1.3 (1.0, 1.9)	1.3 (1.0, 1.8)	1.6 (1.0, 4.0)
HPV11	4.0 (2.4, 6.8)	3.9 (2.3, 6.5)	4.2 (2.4, 7.1)	4.0 (2.3, 6.7)	0.9 (0.2, 4.0)
19-21 years old					
Any High-risk HPV	1.5 (1.3, 1.8)	1.3 (1.2, 1.4)	1.8 (1.5, 2.1)	- [3]	1.6 (1.3, 2.1)
Any other high-risk HPV	1.8 (1.6, 2.2)	1.5 (1.3, 1.7)	2.1 (1.8, 2.5)	1.6 (1.4, 1.8)	1.6 (1.2, 2.1)
Vaccine HPV types					
HPV16 and/or HPV18	0.4 (0.4, 0.5)	0.5 (0.4, 0.6)	0.5 (0.4, 0.7)	0.6 (0.5, 0.7)	0.5 (0.4, 0.8)
HPV16	0.5 (0.4, 0.6)	0.5 (0.4, 0.7)	0.6 (0.5, 0.8)	0.7 (0.5, 0.8)	0.6 (0.4, 0.9)
HPV18	0.3 (0.2, 0.4)	0.3 (0.2, 0.4)	0.3 (0.2, 0.5)	0.3 (0.2, 0.5)	0.5 (0.3, 2.1)

Nonavalent HPV types					
HPV31/HPV33/HPV45/HPV52/HPV58	1.4 (1.1, 1.7)	1.3 (1.1, 1.5)	1.6 (1.3, 1.9)	1.4 (1.2, 1.7)	1.6 (1.2, 2.3)
HPV31/HPV33/HPV45	0.8 (0.6, 1.1)	0.9 (0.7, 1.1)	1.0 (0.8, 1.3)	1.0 (0.8, 1.3)	1.3 (0.7, 2.8)
HPV31	0.4 (0.3, 0.6)	0.4 (0.3, 0.6)	0.5 (0.3, 0.7)	0.5 (0.3, 0.7)	0.7 (0.4, 2.9)
HPV33	1.4 (0.8, 2.4)	1.4 (0.8, 2.3)	1.7 (1.0, 2.8)	1.6 (1.0, 2.7)	2.1 (0.9, 6248.7)
HPV45	1.0 (0.6, 1.5)	1.0 (0.6, 1.5)	1.2 (0.7, 1.9)	1.1 (0.7, 1.8)	1.0 (0.5, 4.3)
HPV52	2.6 (1.8, 3.7)	2.5 (1.7, 3.5)	2.9 (2.0, 4.2)	2.7 (1.9, 3.8)	2.5 (1.5, 5.0)
HPV58	1.0 (0.7, 1.3)	1.0 (0.7, 1.3)	1.0 (0.7, 1.4)	1.0 (0.7, 1.3)	1.2 (0.8, 2.1)
HPV6 and/or HPV11	1.5 (1.1, 2.0)	1.4 (1.1, 1.9)	1.5 (1.1, 2.1)	1.5 (1.1, 2.0)	1.5 (0.9, 2.9)
HPV6	0.9 (0.7, 1.3)	0.9 (0.7, 1.2)	1.0 (0.7, 1.4)	1.0 (0.7, 1.3)	1.2 (0.7, 2.6)
HPV11	10.4 (3.8, 27.8)	9.9 (3.7, 26.5)	10.4 (3.9, 28.1)	9.9 (3.7, 26.6)	2.4 (0.5, 21.6)
22-24 years old					
Any High-risk HPV	1.6 (1.3, 2.0)	1.4 (1.2, 1.5)	1.8 (1.5, 2.2)	1.4 (1.2, 1.6)	1.6 (1.2, 2.2)
Any other high-risk HPV	1.6 (1.3, 2.0)	1.4 (1.2, 1.6)	1.8 (1.4, 2.2)	1.5 (1.3, 1.7)	1.4 (1.0, 1.9)
Vaccine HPV types					
HPV16 and/or HPV18	1.0 (0.8, 1.3)	1.0 (0.8, 1.3)	1.1 (0.8, 1.4)	1.1 (0.9, 1.3)	1.1 (0.8, 1.7)
HPV16	1.3 (1.0, 1.7)	1.2 (1.0, 1.6)	1.3 (1.0, 1.8)	1.3 (1.0, 1.7)	1.3 (0.8, 2.0)
HPV18	0.5 (0.3, 0.7)	0.5 (0.3, 0.7)	0.5 (0.3, 0.8)	0.5 (0.3, 0.8)	0.8 (0.4, 5.6)
Nonavalent HPV types					
HPV31/HPV33/HPV45/HPV52/HPV58	1.4 (1.1, 1.8)	1.3 (1.0, 1.6)	1.5 (1.2, 2.0)	1.4 (1.1, 1.7)	1.5 (1.0, 2.2)
HPV31/HPV33/HPV45	1.1 (0.8, 1.5)	1.1 (0.8, 1.5)	1.1 (0.8, 1.6)	1.1 (0.8, 1.5)	1.3 (0.8, 2.8)
HPV31	0.9 (0.5, 1.5)	0.9 (0.5, 1.5)	1.0 (0.6, 1.7)	1.0 (0.6, 1.6)	1.7 (0.7, -)
HPV33	1.1 (0.6, 2.0)	1.1 (0.6, 2.0)	1.2 (0.7, 2.2)	1.2 (0.7, 2.2)	1.2 (0.5, 15.4)
HPV45	1.0 (0.7, 1.7)	1.0 (0.7, 1.6)	1.1 (0.7, 1.7)	1.0 (0.7, 1.6)	0.8 (0.4, 1.8)
HPV52	2.1 (1.4, 3.1)	2.0 (1.3, 2.9)	2.2 (1.5, 3.4)	2.1 (1.4, 3.1)	1.8 (1.1, 3.7)
HPV58	1.2 (0.7, 2.1)	1.2 (0.7, 2.0)	1.6 (0.9, 2.8)	1.5 (0.9, 2.7)	2.4 (1.2, 20.6)
HPV6 and/or HPV11	1.1 (0.7, 1.8)	1.1 (0.7, 1.8)	1.2 (0.7, 1.9)	1.2 (0.7, 1.8)	1.3 (0.7, 5.0)
HPV6	0.6 (0.4, 1.0)	0.6 (0.4, 1.0)	0.6 (0.4, 1.0)	0.6 (0.4, 1.0)	0.9 (0.4, 558.4)
HPV11	3.9 (1.4, 10.6)	3.8 (1.4, 10.3)	4.0 (1.4, 10.9)	3.9 (1.4, 10.5)	0.9 (0.2, 6.8)

1: *Adjusted for age, venue type and chlamydia positivity*

2: *Adjusted for age, venue type, chlamydia positivity and the assay change between the pre-vaccination and post-vaccination period*

3: *regression model did not converge*

6.4.2. The effect of potential changes in chlamydia screening over time

This HPV surveillance made use of residual specimens taken originally from young women attending for chlamydia screening as part of the National Chlamydia Screening Programme. This is ongoing surveillance started in 2008 and I presented post-vaccination data collected between October 2010 and December 2016 in the second paper in this Chapter (Section 6.3). Over the same time period that this HPV infection surveillance was conducted, there were changes within the Chlamydia Screening Programme. There are two notable changes which could have affected the risk of HPV infection in the HPV infection surveillance population; the number of chlamydia tests performed annually, and the change in recording of chlamydia testing from the NCSP dataset to CTAD (as described in Section 4.2.5). I explore these two changes in more detail and then assess the potential effect that these changes could have on the results presented in the two papers in this section.

Firstly, I considered changes in the total number of chlamydia screening tests performed in England between 2010 and 2016. Assuming one test per person, an estimated 44% of young females were tested for chlamydia in 2010. This compared to an estimated 41% of females tested in 2011[140]. These proportions are not directly comparable to data from 2012 onwards, given the change from NCSP data collection to CTAD data collection. However, a report of CTAD data (published by Public Health England) indicated that between 2012 and 2016, the total number of chlamydia screening tests continued to steadily decline from an estimated 37% of females being screened in 2012 to around 30% in 2016[118]. Data from this report suggested that if a higher number of women were attending for chlamydia screening nationally, it's likely that, on average, the chlamydia positivity among those tested would be lower (as this would include higher and lower risk women). Conversely, if a lower number of women were attending for chlamydia screening nationally, then it's likely these would be higher risk women with a higher proportion positive for

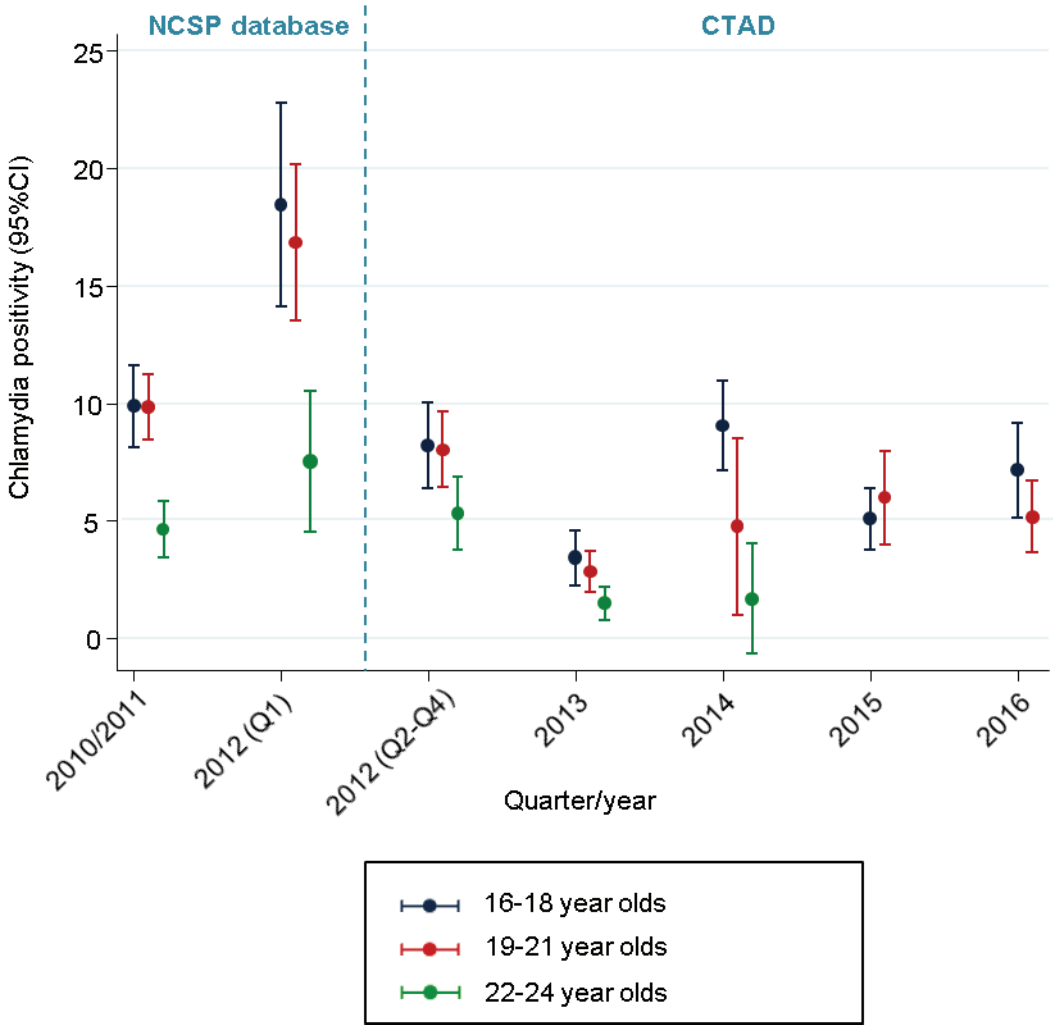
chlamydia infection[118]. Therefore, if the proportion of women attending for chlamydia screening changed over time then this could potentially have affected the results reported in the above papers if there was a change in the risk profile of women included in the surveillance. Specifically, if women attending for chlamydia screening had a varying risk of chlamydia infection over time, they also could have had a varying risk of HPV infection, as the two infections have many shared risk factors. I explore the potential effect of this change at the end of this section.

Secondly, the surveillance population was intended to be restricted to women attending for an opportunistic chlamydia screening test (see Section 4.3.1). Other reasons for attending for a chlamydia test could have been because a woman had symptoms or because she had been notified of a potential risk of chlamydia infection from a partner (i.e. partner notification). Women attending for either of these reasons would have been more likely to have had a chlamydia infection compared to women attending for opportunistic screening, and thus potentially would have been at higher risk of HPV infection. When data were collected using the NCSP dataset (before April 2012), the reason for chlamydia testing was recorded so I was able to confirm that laboratories had correctly sent specimens only from women attending for an opportunistic test and exclude samples from women attending for other reasons; a relatively small proportion of all linked residual specimens (2.1%) were excluded because they were recorded as being taken from a women not attending for opportunistic screening. However, since the introduction of CTAD in April 2012, this information (i.e. the reason for chlamydia testing) was no longer collected (see Section 4.2.5) hence I was not able to confirm that laboratories had exclusively sent samples collected for opportunistic sampling. Therefore, although there was no change in how laboratories were asked to select residual specimens, and although women attending for chlamydia screening would have been unaware of this change in data collection, it is plausible that following April

2012, there were some women included in the surveillance who had not attended for opportunistic chlamydia screening.

Both of the above changes (the decrease in the numbers of women attending for chlamydia screening nationally after 2010 and the potential inclusion of some higher-risk women due to the move from NCSP database to CTAD) could have affected the risk of chlamydia infection in the HPV infection surveillance; most likely leading to a higher chlamydia positivity in later years of the surveillance, as described above. This could in turn have resulted in the inclusion of a higher proportion of HPV positive women in the later years of the post-vaccination period. If this were the case, this would have underestimated the effect of HPV vaccination on HPV prevalence. The effect of these two changes was mitigated in the analysis by adjusting for chlamydia positivity which I used as a proxy for sexual behaviour (the effect of residual confounding by unmeasured sexual behaviour is discussed further in Section 6.4.3). However, to further investigate to what extent these two changes could have affected the results of the HPV infection surveillance, I compared chlamydia positivity over time, as reported in Figure 6.1.

Figure 6.1: Change in chlamydia positivity over time among the HPV infection surveillance population, by age-group



This Figure shows that there were clear changes in the prevalence of chlamydia in the surveillance population over time, with markedly higher chlamydia positivity in the first quarter of 2012 compared to 2010/2011 and compared to the second quarter of 2012 onwards for all age-groups. However, the observed variations over time weren't consistent with the expected effect of either a decline in the number of women attending for chlamydia screening or of the change from the NCSP database to CTAD. There were two laboratories who not only provided specimens with notably higher chlamydia positivity than the other laboratories included in this analysis (14.8% at Leeds and 8.4% at Lewisham compared to 4.7% at all other

laboratories combined) but importantly also had a greater variation in chlamydia positivity over time. Figure 6.2 shows the chlamydia positivity among specimens included in the HPV infection surveillance after excluding specimens from Leeds and Lewisham laboratories. After these exclusions, there was far less variation in the prevalence of chlamydia positivity over time, although there was a small increase among the younger two age-groups after 2014.

As a sensitivity analysis, I calculated changes in HPV prevalence after excluding specimens from the Leeds and Lewisham laboratories. The results excluding these two laboratories were very similar to those from the main analysis; declines in the prevalence of HPV16 and/or 18 were seen for all age-groups and there was evidence of a reduction in HPV31/33/45 prevalence in the younger two age-groups. The prevalence of other high-risk HPV types remained relatively stable within the post-vaccination period (Figure 6.3). The similarity of these results to the main analysis presented in the second paper of this Chapter (Section 6.3) reassures that changes in chlamydia positivity due to variations over time in two geographical areas (served by two laboratories) did not substantially affect the results presented in this Chapter. I also include in Appendix F, the same Figure restricted to women testing negative for chlamydia. As expected, the HPV prevalence is slightly lower but the pattern of declines over time is again similar to the main analysis; numbers were too small to repeat this analysis restricted to women testing positive for chlamydia. In the next section (Section 6.4.3), are the results of further exploration of the possibility that other changes in sexual behaviour over time (not accounted for by adjustment in chlamydia positivity) could potentially bias the results of the HPV infection surveillance.

Figure 6.2: Change in chlamydia positivity over time among the HPV infection surveillance population, by age-group (excluding specimens from Leeds laboratory and Lewisham laboratory)

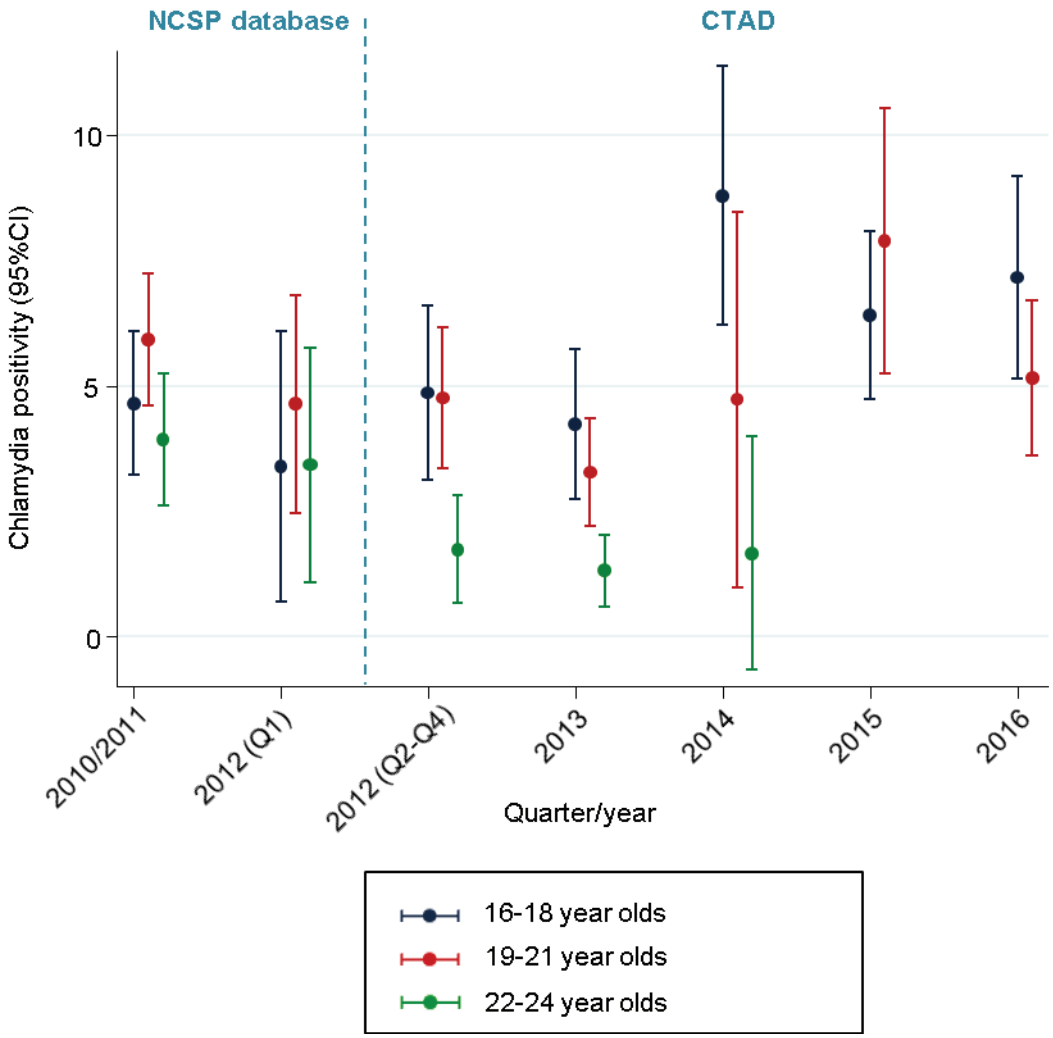
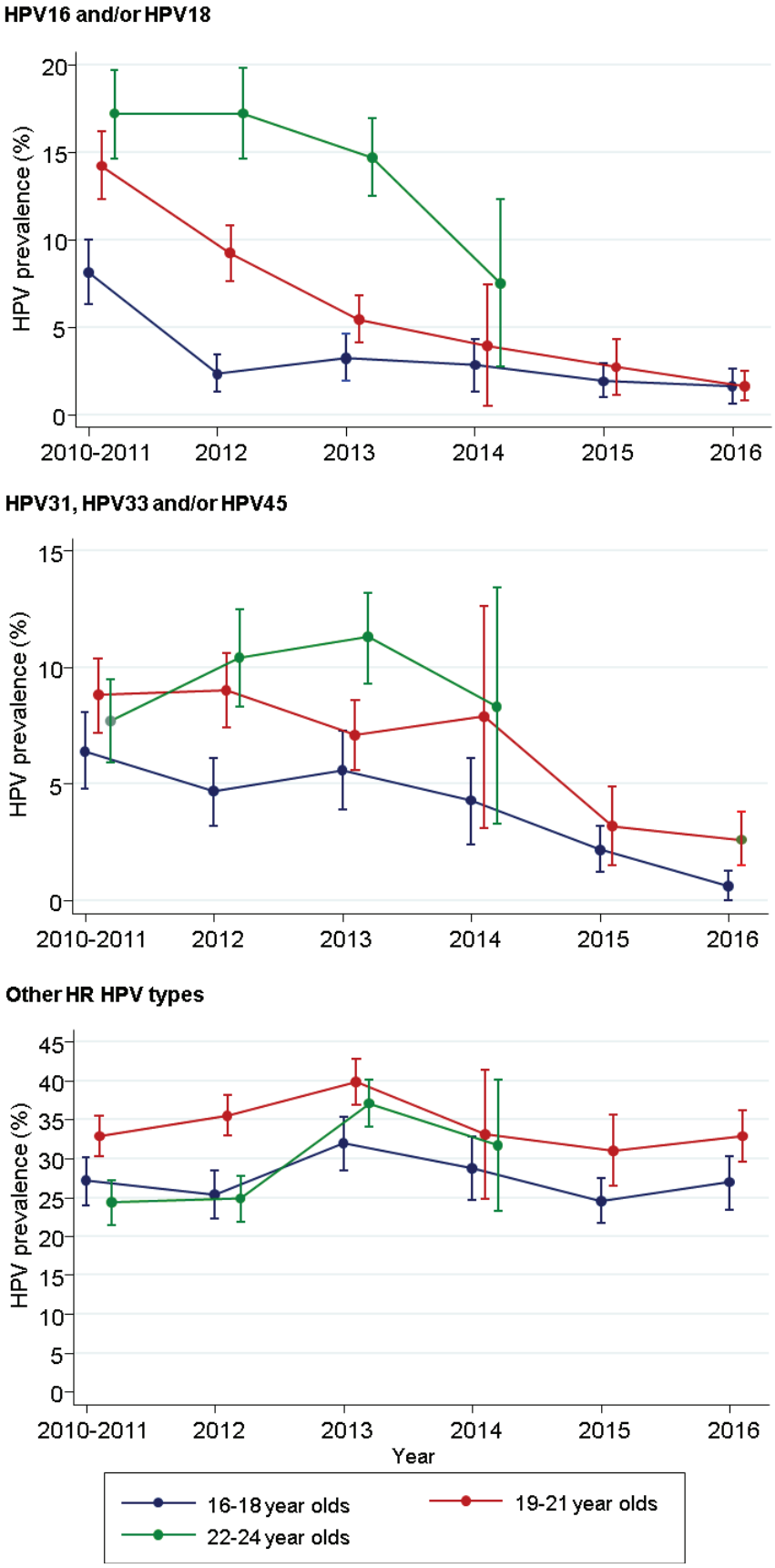


Figure 6.3: Prevalence of HPV infection by year of sample collection (excluding specimens from Leeds laboratory and Lewisham laboratory)



6.4.3. Potential bias due to missing data and unmeasured confounding variables

The NCSP and CTAD datasets had a considerable amount of missing data for some variables of interest to HPV surveillance. I described this in Section 4.2.5 (Table 4.1) for the general datasets and I explore this specifically for the specimens included in the HPV surveillance in Table 6.3, below.

There was no (or very little) missing data for the venue where the sample was collected, for age or for chlamydia positivity and hence these could be adjusted for in the analysis. Data on the number of sexual partners has not been available since 2012 with the introduction of CTAD. Therefore, this variable could not be considered in analyses within the post-vaccination period. Unfortunately, data on quintile of deprivation was not available for pre-vaccination specimens. Within the post-vaccination period the percentage of specimens with missing IMD varied from 2% to 26% across different years. Due to the variation in IMD data completeness over time, I did not adjust for IMD in the calculation of the prevalence ratio associated with estimated vaccination coverage (Table 3 in the second paper of this Chapter; Section 6.3) or the calculation of the vaccine effectiveness (Table 4 in the second paper of this Chapter; Section 6.3) Here, I have included a complete case analysis adjusted for quintile of IMD below (Table 6.4 for the prevalence ratio associated with vaccination coverage and Table 6.5 for the vaccine effectiveness). The results for both these analyses were very similar to those without adjustment for IMD. There was a much higher proportion of missing data for ethnicity and this also varied considerably over time (between 12% and 95%). Therefore it was not appropriate to conduct an analysis incorporating only specimens with non-missing ethnicity and this variable was not included in multivariable analyses. I discuss the implications of this below.

Table 6.3: Percentage of specimens included in the HPV surveillance with missing data in the NCSP dataset (2008-March 2012) or in CTAD (April 2012-2016), stratified by year of specimen collection

	Year of specimen collection						
	2008	2010/2011	2012	2013	2014	2015	2016
Socio-demographic data							
Age	0%	0%	0%	0%	0%	0%	0%
Ethnicity	12%	25%	38%	95%	83%	50%	55%
Quintile of IMD[1]	100%	2%	6%	16%	7%	5%	26%
>1 sexual partner in previous 12 months[2]	19%	55%	63%	100%	100%	100%	100%
Any new sexual partner in previous 3 months[2]	12%	13%	22%	100%	100%	100%	100%
Details of chlamydia test							
Sample collection venue	0%	0%	0%	0%	0%	0%	0%
Result of chlamydia test	1%	<1%	0%	0%	0%	0%	0%

1: Index of multiple deprivation (IMD) was derived from the postcode of residence. This was not linked prior to anonymisation for pre-vaccination specimens in 2008 hence these data are not available for this year

2: Sexual behaviour data were collected in the NCSP dataset but not in CTAD

Table 6.4: Complete case analysis for prevalence ratio for HPV associated with estimated HPV vaccination coverage including additional adjustment for IMD, by age-group and HPV type (specimens collected 2010-2016)

	Prevalence ratio associated with estimated vaccination coverage		
HPV type	Unadjusted (95% CI)	Adjusted[1] (95%CI)	IMD adjusted[2] (95%CI)
16-18 years old	n=5,260	n=5,260	n=5,260
Any High-risk HPV	0.7 (0.6, 0.8)	-	-
Any other high-risk HPV	0.7 (0.6, 0.9)	-	-
Vaccine HPV types			
HPV16 and/or HPV18	0.1 (0.1, 0.2)	0.2 (0.1, 0.3)	0.2 (0.1, 0.3)
HPV16	0.1 (0.1, 0.2)	0.2 (0.1, 0.3)	0.2 (0.1, 0.3)
HPV18	0.1 (0.0, 0.3)	0.1 (0.0, 0.3)	0.1 (0.1, 0.4)
Nonavalent HPV types			
HPV31/HPV33/HPV45/HPV52/HPV58	0.4 (0.3, 0.6)	0.5 (0.4, 0.8)	0.6 (0.4, 0.8)
HPV31/HPV33/HPV45	0.2 (0.2, 0.4)	0.3 (0.2, 0.5)	0.3 (0.2, 0.5)
HPV31	0.1 (0.1, 0.4)	0.2 (0.1, 0.5)	0.2 (0.1, 0.6)
HPV33	0.3 (0.1, 0.5)	0.3 (0.1, 0.6)	0.3 (0.1, 0.6)
HPV45	0.2 (0.1, 0.6)	0.3 (0.1, 0.7)	0.3 (0.1, 0.7)
HPV52	0.7 (0.4, 1.1)	1.0 (0.6, 1.8)	1.1 (0.6, 1.8)
HPV58	0.5 (0.2, 0.9)	0.7 (0.3, 1.5)	0.7 (0.3, 1.5)
HPV6/11	0.8 (0.5, 1.2)	0.9 (0.6, 1.4)	0.9 (0.6, 1.4)
HPV6	1.1 (0.6, 1.9)	1.4 (0.7, 2.6)	1.4 (0.7, 2.6)
HPV11	0.6 (0.3, 0.9)	0.6 (0.4, 1.0)	0.6 (0.4, 1.1)

19-21 years old	n=5,460	n=5,460	n=5,460
Any High-risk HPV	0.9 (0.8, 0.9)	1.0 (0.9, 1.2)	-
Any other high-risk HPV	1.0 (0.9, 1.1)	1.0 (0.9, 1.2)	1.0 (0.9, 1.2)
Vaccine HPV types			
HPV16 and/or HPV18	0.2 (0.2, 0.3)	0.3 (0.2, 0.4)	0.3 (0.2, 0.4)
HPV16	0.3 (0.2, 0.4)	0.3 (0.2, 0.5)	0.3 (0.2, 0.5)
HPV18	0.1 (0.1, 0.3)	0.1 (0.1, 0.3)	0.1 (0.1, 0.3)
Nonavalent HPV types			
HPV31/HPV33/HPV45/HPV52/HPV58	0.7 (0.6, 0.8)	0.8 (0.6, 0.9)	0.8 (0.6, 0.9)
HPV31/HPV33/HPV45	0.4 (0.3, 0.6)	0.5 (0.4, 0.7)	0.5 (0.4, 0.7)
HPV31	0.4 (0.2, 0.6)	0.5 (0.3, 0.9)	0.5 (0.3, 0.9)
HPV33	0.5 (0.3, 0.9)	0.6 (0.3, 1.0)	0.6 (0.3, 1.0)
HPV45	0.4 (0.2, 0.6)	0.4 (0.2, 0.7)	0.4 (0.2, 0.7)
HPV52	0.9 (0.7, 1.1)	0.9 (0.7, 1.2)	0.9 (0.7, 1.2)
HPV58	0.9 (0.6, 1.3)	0.9 (0.6, 1.3)	0.9 (0.6, 1.3)
HPV6/11	1.2 (0.9, 1.5)	1.1 (0.8, 1.5)	1.1 (0.8, 1.5)
HPV6	1.0 (0.7, 1.5)	0.9 (0.6, 1.4)	0.9 (0.6, 1.4)
HPV11	1.4 (1.0, 2.0)	1.3 (0.9, 2.0)	1.3 (0.9, 2.0)
22-24 years old	n=3,348	n=3,348	n=3,348
Any High-risk HPV	1.0 (0.8, 1.2)	1.0 (0.8, 1.3)	1.0 (0.8, 1.3)
Any other high-risk HPV	1.3 (1.0, 1.6)	1.3 (1.0, 1.7)	1.3 (1.0, 1.7)
Vaccine HPV types			
HPV16 and/or HPV18	0.3 (0.2, 0.6)	0.3 (0.1, 0.6)	0.3 (0.2, 0.6)
HPV16	0.3 (0.2, 0.6)	0.3 (0.1, 0.6)	0.3 (0.1, 0.6)
HPV18	0.4 (0.1, 1.7)	0.3 (0.1, 1.6)	0.3 (0.1, 1.5)

Nonavalent HPV types			
HPV31/HPV33/HPV45/HPV52/HPV58	1.1 (0.8, 1.6)	1.2 (0.8, 1.7)	1.2 (0.8, 1.7)
HPV31/HPV33/HPV45	0.6 (0.3, 1.2)	0.6 (0.3, 1.3)	0.6 (0.3, 1.3)
HPV31	0.6 (0.2, 2.2)	0.5 (0.1, 2.0)	0.5 (0.1, 2.0)
HPV33	1.0 (0.3, 2.9)	0.9 (0.3, 2.6)	0.9 (0.3, 2.7)
HPV45	0.4 (0.1, 1.4)	0.5 (0.2, 1.8)	0.5 (0.2, 1.9)
HPV52	1.6 (1.0, 2.5)	1.7 (1.0, 2.9)	1.7 (1.0, 2.8)
HPV58	2.1 (1.0, 4.5)	2.2 (0.9, 5.1)	2.2 (0.9, 5.1)
HPV6/11	2.1 (1.1, 3.9)	1.8 (0.9, 3.4)	1.8 (0.9, 3.4)
HPV6	2.0 (0.8, 5.3)	1.3 (0.5, 3.7)	1.3 (0.5, 3.6)
HPV11	2.1 (1.0, 4.8)	2.1 (0.9, 5.0)	2.1 (0.9, 5.0)

1: Adjusted for age, venue type and chlamydia positivity

2: Adjusted for age, venue type, chlamydia positivity and quintile of IMD

Table 6.5: Complete case analysis for vaccine effectiveness comparing HPV infection in the post-vaccination period including additional adjustment for IMD, by age-group

	VE[1] (full vaccination) (95% CI)	VE[2] (full vaccination) (95% CI)
Younger HPV vaccination cohorts[3] combined	n=1,124	n=1,124
HPV 16/18 with or without other HR types	79.9 (50.0, 91.9)	80.7 (51.8, 92.2)
Non-vaccine type(s) with or without HPV 16/18		
HPV 31/33/45	60.3 (20.1, 80.3)	60.1 (19.7, 80.2)
HPV 31/33/45/52/58	21.1 (-26.5, 50.8)	20.2 (-28.0, 50.2)
HPV6/11	12.0 (-63.6, 52.6)	11.8 (-63.8, 52.5)
Older catch-up HPV vaccination cohorts	n=860	n=860
HPV 16/18 with or without other HR types	51.5 (24.8, 68.8)	53.7 (27.6, 70.3)
Non-vaccine type(s) with or without HPV 16/18		
HPV 31/33/45	38.9 (-0.8, 63.0)	37.3 (-3.8, 62.1)
HPV 31/33/45/52/58	20.8 (-3.7, 39.6)	19.4 (-5.9, 38.6)
HPV6/11	10.9 (-40.0, 43.3)	9.8 (-42.2, 42.8)

1: Adjusted for age, venue type and chlamydia positivity

2: Adjusted for age, venue type, quintile of IMD and chlamydia positivity

3: Including routine cohorts and younger vaccination cohorts (i.e. all women offered vaccination at age 15 or younger)

Estimates of changes in HPV prevalence over time among women attending for chlamydia screening could be subject to confounding if there are changes in related demographics or sexual behaviour of the screened population over the same time period. As described in the previous section, I attempted to account for these changes by including relevant available variables in multivariable regression models (age, chlamydia positivity and testing venue). However, other unrecorded population changes over time which were related to HPV prevalence may have confounded the estimated association between HPV vaccination coverage and trends in HPV prevalence. I discuss below some of the known confounding variables which could not be included in our analyses.

Sexual behaviour: In the earlier years of this HPV surveillance, some data were available on the proportion of women with multiple sexual partners in the previous 12 months and the proportion of women with at least one new sexual partner within the last 3 months. These data variables were collected from clinics at the time of the chlamydia test. Unfortunately, with the change to data collection from laboratories in April 2012, these data could no longer be collected so these variables could not be included in this analysis. I compared changes in sexual behaviour over time (up to April 2012) in the analysis I conducted prior to the start of this PhD[126] but the interpretation was limited by the high proportion of missing data. As discussed above, in the updated analyses included in this Chapter I was also able to adjust for chlamydia positivity as a proxy for sexual behaviour. However, other changes in sexual behaviour may not have been addressed by adjustment by chlamydia positivity alone. The expectation, and concern, when interpreting these results, is that those with higher risk sexual behaviour would have a lower HPV vaccination uptake but a higher risk of HPV infection (i.e. a positive confounder) Therefore, by not including sexual behaviour in the analysis, the association between vaccination

coverage and HPV prevalence may be overestimated. I explore variations in HPV vaccination uptake in different population subgroups in Chapter 8.

Ethnicity: As previously discussed, the proportion of specimens with missing ethnicity was considerable hence this variable was not included in the analysis. I explore later in this thesis whether women of non-white ethnicity have lower HPV vaccination uptake (Section 8.4). There was some evidence of lower vaccination coverage in black women and women of mixed ethnicity although this was not clear after adjustment for other variables. Others have shown that the prevalence of HPV is higher in black women but lower in Asian women (Section 2.3). As with sexual behaviour, if black women were likely have lower vaccination uptake and higher HPV prevalence, lack of adjustment would overestimate the association between vaccination coverage and HPV prevalence. For Asian women, the reverse is true if these women had lower vaccination uptake but also lower HPV prevalence. This could have potentially underestimated the association between vaccination and HPV prevalence although the number of specimens from Asian women was small so this is likely to have less impact.

Smoking status: Smoking has been shown to be associated with increased HPV infection (Section 2.3). The work to explore variations in HPV vaccination coverage in Chapter 7 and Chapter 8 did not consider smoking status. However, others have shown lower vaccination uptake among women who smoked[141]. As with previous factors, smoking could be a positive confounder and hence not collecting and adjusting for this could have overestimated the effect of vaccination on HPV prevalence.

6.4.4. Misclassification of HPV vaccination status

Calculation of vaccine effectiveness and estimation of herd protection effects in the second paper of this chapter (Section 6.3) were only possible for women for whom

HPV vaccination status was collected in the HPV infection surveillance. In this section, I discuss three limitations of this HPV vaccination data collection, specifically; (i) vaccination status was only available for a minority of women in this surveillance; (ii) the vast majority of vaccination status data were for samples from one laboratory (Cornwall); (iii) the vast majority of vaccination status data were collected from CHIS.

Firstly, vaccination status was only available for 21% of eligible women included in this analysis. Vaccination status data were often missing for an entire geographical area (either because local laboratories sending residual specimens did not have access to NHS number or because it was not possible to link with local CHIS data; Table 5.3). The concern, when considering the results of the HPV vaccine effectiveness analysis, is that the women without vaccination records differed in their risk of HPV infection compared to women with vaccination records. In Table 2 of the second paper in this Chapter (Section 6.3), I compared patient characteristics of women with and without vaccination status and of vaccinated and unvaccinated women. There was a higher proportion of black women with a known vaccination status compared to those with unknown vaccination status. There were also marked differences in the proportion of women attending general practices and family planning venues between those with and without known vaccination status; although this was most likely due to the greater availability of NHS numbers at some venues compared to others. As described in the paper, the chlamydia positivity was slightly higher among unvaccinated women. There was also a higher proportion of black women and women attending family planning clinics for unvaccinated women compared to vaccinated women. As the majority of vaccination records (>80%) were for specimens from one testing laboratory in Cornwall (discussed in more detail in the next paragraph), I repeated this analysis restricted to results from the Cornwall laboratory to see if this mitigated some of

these differences (Table 6.6, below). It is encouraging that within Cornwall, the differences in chlamydia positivity were less marked. As above, the differences in the proportions from each sample collection venue in those with and without vaccination status was largely due to certain venues having access to NHS numbers, enabling linkage to vaccination records. However, within Cornwall, the proportion from each recruitment venue was similar for vaccinated and unvaccinated women. There was very little complete data on ethnicity from samples collected from the Cornwall laboratory, and differences by ethnicity could not be considered.

Table 6.6: Patient characteristics of vaccinated and unvaccinated women, and of women with and without recorded vaccination status, in the post-vaccination period (restricted to specimens collected from the Cornwall laboratory)

	Known vaccination status			
	Vaccinated (n=1,647)	Unvaccinated (n=277)	Total with known vaccination status (n=1,924)	Unknown vaccination status (n=628)
Age - years [data completeness]	[100%]	[100%]	[100%]	[100%]
16-18 years	853 (51.8%)	78 (28.2%)	931 (48.4%)	270 (27.1%)
19-21 years	718 (43.6%)	179 (64.6%)	897 (46.6%)	413 (65.8%)
22-24 years	76 (4.6%)	20 (7.2%)	96 (5.0%)	45 (7.2%)
Ethnicity[1] [data completeness]	[2%]	[2%]	[2%]	[0%]
Sample collection venue [data completeness]	[100%]	[100%]	[100%]	[100%]
General practice	1,416 (86.0%)	232 (83.8%)	1,648 (85.7%)	407 (64.8%)
Family planning (Community Sexual Health Services; CaSH)	207 (12.6%)	36 (13.0%)	243 (12.6%)	198 (31.5%)
Youth clinic	24 (1.5%)	9 (3.2%)	33 (1.7%)	23 (3.7%)
Chlamydia positivity [data completeness]	6.9% [100%]	7.9% [100%]	7.0% [100%]	6.4% [100%]

1: Numbers too small to include

Secondly, more than 80% of vaccination records were for specimens from one testing laboratory in Cornwall. Vaccination in Cornwall differed from elsewhere in England as the vaccination was offered and delivered in general practice rather than in schools. This could potentially affect the estimation of vaccine effectiveness if this method of delivery was suboptimal (Section 6.3); I briefly discussed whether vaccination at primary care may mean that women were less likely to receive the vaccine doses within the recommended schedule as they would need to be followed up individually rather than having mop-up sessions at schools. Women included in the HPV infection surveillance would have been vaccinated using the three dose schedule for Cervarix; the second dose given between one and two and a half months after the first dose; the third dose given between five and 12 months after the first dose[121]. If second or third doses were given outside of the recommended schedule, this could have resulted in a lower estimate of vaccine effectiveness. There was no evidence that the proportion of fully vaccinated women in the HPV infection surveillance vaccinated within the recommended schedule was different at the Cornwall laboratory compared to other laboratories (Table 6.7). However, the numbers of women with vaccination status from the other laboratories were small; therefore, to explore this further, I recalculated the vaccine effectiveness after excluding women who received 3-doses of the vaccine outside of the recommended schedule. There was a small difference in the HPV16/18 vaccine effectiveness in all fully vaccinated women versus women vaccinated within the recommended schedule (from 82.0% to 85.1% for women vaccinated at a younger age; Table 6.8). This suggests that collection of vaccination status largely from women vaccinated at general practice may have led to slightly lower vaccine effectiveness estimates.

Table 6.7: Proportion of women recorded as fully vaccinated who were vaccinated within the recommended schedule[2]

	Cornwall laboratory		All other laboratories	
	Younger vaccination cohorts (vaccinated <16 yrs old) (n=992)	Older vaccination cohorts (vaccinated ≥16 yrs old) (n=546)	Younger vaccination cohort (vaccinated <16 yrs old) (n=184)	Older vaccination cohorts (vaccinated ≥16 yrs old) (n=68)
Unknown[1]	0	0	24	39
Vaccinated within recommended schedule[2]	84.4% (82.1, 86.7)	83.2% (79.7, 86.2)	79.4% (72.3, 85.4)	82.8% (64.2, 94.2)
Vaccinated outside recommended schedule[2]	15.5% (13.3, 17.9)	16.8% (13.8, 20.3)	20.6% (14.6, 27.7)	17.2% (5.8, 35.8)

1: *Vaccination data from Lewisham laboratory did not include dates of HPV vaccination*

2: *the second dose given between one and two and a half months after the first dose; the third dose given between five and 12 months after the first dose*

Table 6.8: Vaccine effectiveness[1] comparing HPV infection in the post-vaccination period, for women with and without vaccination delivered within the recommended schedule

	VE (full vaccination) (95% CI)	VE[2] (full vaccination within recommended schedule) (95% CI)
Younger HPV vaccination cohorts[3] combined	n=1,293	n=1,093
HPV 16/18 with or without other HR types	82.0 (60.6, 91.8)	85.1 (62.5, 94.1)
Non-vaccine type(s) with or without HPV 16/18		
HPV 31/33/45	54.3 (8.6, 77.2)	61.0 (26.7, 79.2)
HPV 31/33/45/52/58	16.4 (-30.9, 46.5)	23.6 (-17.1, 50.1)
HPV6/11	26.5 (-26.8, 57.4)	30.3 (-17.2, 58.5)
Older catch-up HPV vaccination cohorts	n=903	n=779
HPV 16/18 with or without other HR types	48.7 (20.8, 66.8)	54.4 (26.4, 71.8)
Non-vaccine type(s) with or without HPV 16/18		
HPV 31/33/45	36.7 (-3.4, 61.2)	44.8 (8.8, 66.6)
HPV 31/33/45/52/58	20.6 (-3.5, 39.1)	21.7 (-2.9, 40.5)
HPV6/11	18.8 (-24.6, 47.1)	8.0 (-42.2, 40.4)

1: Adjusted for age, venue type and chlamydia positivity

2: the second dose given between one and two and a half months after the first dose; the third dose given between five and 12 months after the first dose

3: Including routine cohorts and younger vaccination cohorts (i.e. all women offered vaccination at age 15 or younger)

Finally, vaccine effectiveness in the second paper in this Chapter (Section 6.3) was calculated using HPV vaccination records largely collected from CHIS data. This could be a concern if vaccination records in CHIS were incomplete or inaccurate; as suggested in Section 5.1.1, this is particularly plausible for vaccinations administered outside of school settings. In the results of the HPV infection surveillance in the second paper in this chapter (Section 6.3), 83% of women with vaccination data were from the laboratory in Cornwall (where women were vaccinated in general practice and the results reported to CHIS) and a further 8% were from women who were over 16 years old when the National HPV Immunisation Programme was introduced (also likely offered vaccination in general practices).

I described in Section 5.3 a validation study which compared CHIS data with GP data in Cornwall (the results are shown in Table 5.7). Here, I explore the implications of misclassification of vaccination status using CHIS data. If this misclassification was non-differential (i.e. incorrect recoding of a women's vaccination status was unrelated to HPV positivity) then the bias would likely have underestimated vaccine effectiveness. However, it is possible that there was differential misclassification (for example, if those with inaccurate recording were from higher-risk populations). If women recorded as fully vaccinated on CHIS were truly vaccinated but there was some misclassification of unvaccinated women as vaccinated, then this would underestimate the vaccine effectiveness if misclassified women were at higher risk for HPV infection compared to women correctly classified as unvaccinated. Conversely, if women classified as unvaccinated on CHIS were truly unvaccinated but there was some misclassification of vaccinated women as unvaccinated, then this would overestimate the vaccine effectiveness if misclassified women were higher-risk for HPV infection. Reassuringly, results from the validation study (as presented in Section 5.4.2) show relatively high agreement

for fully vaccinated and unvaccinated women. There was less agreement for partially vaccinated women (Table 5.7) but as these represented a relatively small proportion of women in the HPV infection surveillance (~5% of those with a known vaccination status) this had less potential to bias the vaccine effectiveness estimates.

In this section, I have added to the discussion of the second paper of this chapter (Section 6.3) about the limitations of HPV vaccination status data used in the HPV infection surveillance. I have discussed the potential misclassification bias of inaccurate vaccination status data in Cornwall, acknowledging that there may well be different misclassification bias for vaccination data from other areas (for example, among the small number of records with self-reported vaccination status collected on the chlamydia test request form). The fact that HPV vaccination status data were only available from a relatively small proportion of women, largely from one particular area, is not overly concerning unless there is reason to believe that the vaccine effectiveness results are not generalisable to the rest of England. Despite this, in an ideal scenario, the HPV infection surveillance presented in the second paper in this chapter (Section 6.3) would have included HPV vaccination status data for all women. The difficulty I have experienced in obtaining accurate vaccination status data for this surveillance highlights the need for a single standardised dataset of HPV vaccination records regardless of where vaccination is offered and delivered. I discuss this further in the main discussion of this thesis (Sections 9.2.2 and 9.4).

6.5. Potential increases in non-vaccines HPV types

In the next section, I discuss the interpretation of changes in non-vaccine HPV types in the post-vaccination period and describe methods for a study which could theoretically be conducted if there was any suggestion of potential type-replacement in the future.

6.5.1. Background

As described in Chapter 3, there have been concerns that reductions in the HPV vaccine types will lead to other non-vaccine HPV types filling this ecological niche and becoming more common, known as type-replacement. Surveillance to monitor the likely impact of the HPV vaccination programme should remain vigilant for such increases. If there was type-replacement then we would expect there to be increases in the prevalence of non-vaccine HPV types between the pre-vaccination and post-vaccination period. Importantly, we would also expect increases within the post-vaccination period, associated with decreases in vaccine types.

The interpretation of changes in non-vaccine types in this surveillance are not straight forward and should be interpreted with care. In the analysis presented in the first paper in this analysis (Section 6.2), there was no evidence of a decrease in the closely-related HPV types (HPV31, 33 and 45) between the pre- and post-vaccination period. For the other non-vaccine high-risk HPV types there was an observed increase between the pre- and post-vaccination periods. However, when considering these changes in non-vaccine types, it is important to take into account the change in the HPV assay between the pre- and post-vaccination period. As reported in Section 4.4.2, a validation study comparing the two assays demonstrated that positivity was similar for the vaccine types but there were some differences in sensitivity and specificity for some non-vaccine types. I adjusted in this analysis for the change in sensitivity and specificity after the new assay was introduced, but the uncertainty surrounding some sensitivity estimates, particularly for the rarer types, led to wide confidence intervals for these odds ratios. To mitigate the limitation of the change in the assay between the pre- and post-vaccination periods, the more recent analyses in Section 6.3 (the second paper in this Chapter) focussed on changes within the post-vaccination period and their association with vaccination coverage. This analysis showed a decrease in the prevalence of HP31,

33 and 45 over time within the post-vaccination period and vaccine effectiveness in younger vaccinated cohorts against these HPV types of 54% (95% CI; 9% to 77%). The prevalence of the other high-risk risk types has remained relatively stable within the post-vaccination period, if not slightly declining in more recent years.

In summary, if increases in non-vaccine types were associated with higher vaccination coverage and/or decreasing HPV16/18 prevalence then this would be of concern. However, the results from Chapter 3 and Section 6.3 are reassuring that there does not appear to be any evidence of increases in non-vaccine HPV types due to type-replacement.

6.5.2. What is the unmasking effect?

Broad-spectrum assays, such as those used in both the pre- and post-vaccination periods of this national surveillance, can have lower accuracy to detect type-specific HPV infections in the presence of multiple infections (Section 2.7.3). As explained previously, HPV types at low concentration (i.e. low copy number) are particular vulnerable if present alongside an HPV type at high concentration (i.e. high copy number) as the types with lower copy numbers may well be “masked” by the other HPV type.

This is of concern for our HPV surveillance due to the reductions in vaccine types (HPV16 and HPV18) following the introduction of the HPV vaccination programme. In the absence of these vaccine types, the broad spectrum assays may have identified other HPV types which would have otherwise been masked (i.e. in the pre-vaccination period). Therefore, this unmasking effect may have made it appear that there was an increase in non-vaccine high-risk HPV types which was entirely artificial.

6.5.3. Future approaches to quantify the unmasking effect

Rationale

In the analyses conducted as part of this thesis, there was no clear evidence of type-replacement or unmasking. Despite this lack of evidence it is still important to remain vigilant for potential increases in non-vaccine types. Any increases due to type-replacement or unmasking are likely to present with similar results as both would be associated with decreases in HPV16/18 infection. However, the implications of type-replacement (i.e. true increases in non-vaccine types) vs. unmasking (i.e. the false impression of increasing non-vaccine types) are clearly very different for public health policy. Therefore, if there were increases in non-vaccine high-risk types, it would be important to quantify the potential unmasking effect so that these increases can be accurately interpreted. Others have estimated an unmasking effect in different settings[18, 142]. However, this effect will be dependent on the sample type and the HPV assay used for testing. I briefly describe below a proposed approach to quantify the potential proportion of non-vaccine HPV infections which could be masked by HPV16/18 infection when using the broad-spectrum HPV assays used for surveillance in England. To quantify the level of unmasking, samples which tested positive for HPV16 and/or 18 but negative for other high-risk HPV-types using the broad spectrum assay could be retested for the other high-risk types using a type-specific PCR. Type-specific PCR tests are not subject to the same issue of unmasking as the primers used are single target and hence provide a more sensitive test in the presence of multiple infections[17, 143].

In practice, I have not proposed that such a study should be conducted since there is little evidence to date of increases in non-vaccine types since the introduction of the HPV vaccine in England (hence costs for additional testing cannot be justified to explore a theoretical problem which has not yet materialised). The below is a brief description of an approach which could be considered if there were ever evidence of

increases in the prevalence of non-vaccine HPV types which were associated with decreasing HPV16/18 infection.

Theoretical choice of non-vaccine type

If there was evidence of type-replacement then ideally, any future study to investigate this would involve testing for all non-vaccine high-risk HPV types. However, in practice, it is unlikely that there would be sufficient residual specimen to conduct this testing and, even if there was sufficient material, the cost of doing this would be prohibitive. Therefore, a more reasonable approach would be to select a single non-vaccine type which can be used to investigate unmasking and the results then broadly applied to other types. A suitable non-vaccine type should meet the following criteria:

(i) Be a type of the same species as one of the two HPV vaccine types as these types are more likely to fill the ecological niche of HPV16 or HPV18 (i.e. either α7 species related to HPV16, or α9 species related to HPV18; Table 2.1).

(ii) Have high concordance between the pre-vaccination and post-vaccination assay so that this does not complicate the quantification with the need for further adjustment for the change in assay between the two periods.

The non-vaccine type would also, ideally (but not essentially):

(iii) Have a higher prevalence in the post-vaccination period vs. the pre-vaccination period; as stated in the rationale above, there is little evidence of increases in non-vaccine types to date. However, this would be an ideal criterion when considering such a study.

(iv) Have no evidence from clinical trials of cross-protection (which would lead to a reduction of the HPV type in vaccinated women)

A summary of these criteria for non-vaccine high-risk types included in the HPV infection surveillance are shown in Table 6.9 below. None of the HPV types meet all of the above criteria but four high-risk types meet all except for one non-essential criteria (HPV33, HPV39, HPV52 and HPV58). I therefore considered HPV58 as a theoretical example for the below methodology.

Methods

Residual samples, previously testing negative for HPV58 by a broad spectrum assay, could be selected for re-testing with type-specific PCR. Samples which were positive and negative for HPV16 and/or HPV18 by the same broad spectrum assay should be re-tested.

Which residual specimens to include for type-specific testing would depend on how increases in non-vaccine types manifest. If there were an increase in non-vaccine types between the pre- and post-vaccination periods then the approach would be to re-test pre-vaccination specimens which were negative for the candidate non-vaccine type using the broad-spectrum assay. Estimates of pre-vaccination prevalence of the candidate type could then be adjusted upwards to account for any effect of masking. If there were increases in non-vaccine types within the post-vaccination period then the approach would be to re-test earlier post-vaccination specimens which were negative for the candidate non-vaccine type using the broad-spectrum assay.

The laboratory methods for the two broad spectrum assays have been described previously (prior to this PhD[19] for the pre-vaccination specimens, and in Section 4.3.9 for post-vaccination specimens). As this is a theoretical approach to explore

changes in non-vaccine types, I am not able to describe the laboratory methods of a suitable type-specific test. However, generally, the approach would be to re-test residual specimens (blind to the original HPV16/18 status) using a type-specific PCR for HPV58.

Power to detect a difference in HPV prevalence

The prevalence of HPV58 in samples originally HPV16/18 positive vs. those HPV16/18 negative would be compared using logistic regression to calculate odds ratios and associated 95% confidence intervals.

Table 6.10 below gives the minimum detectable difference of HPV prevalence which could be identified for 80%, 85% and 90% power, a type-specific prevalence of HPV58 of 2.5% and 5% in HPV16/18 negative specimens and two fixed samples sizes based on data collected to the end of 2016; the first if testing pre-vaccination specimens (n=369 in each group) and the second if testing post-vaccination specimens (n=1,193 in each group). These odds ratios are all relatively large and may exceed expectations of any potential effect of masking. This suggests that if this study were to be conducted then additional sources of residual specimens may need to be added to increase the sample size. This is ongoing surveillance so, over time, the number of post-vaccination specimens will increase.

This concludes the first part of this PhD (Chapters 3 to 6) which considered changes in the prevalence of HPV infection. I will discuss the implications of the results presented in this chapter in the final chapter of this thesis (Chapter 9). In the next Chapter (Chapter 7), I describe the data sources and methods of surveillance to monitor the serological response to the vaccine HPV types (as a biological marker to estimate vaccination coverage). I also describe the methods of a nested case-control study designed to estimate the potential effect of the bivalent HPV vaccine on the incidence of genital warts.

Table 6.9: Selection of non-vaccine types for type-specific HPV testing to quantify unmasking effect

(entries in red do not meet essential criteria; entries in orange do not meet desirable criteria)

HPV type	Species	Evidence for cross protection	Concordance in validation study (kappa)	OR (pre- vs. post-vaccination) in 16-18 year olds[1]	Number of specimens pre-vaccination 16/18 positive, candidate type negative	Number of specimens post-vaccination 16/18 positive, candidate type negative
31	α9	**Yes**	0.77	**0.5 (0.2-3.2)**	358	1,231
33	α9	**Yes**	0.75	1.1 (0.5-3.7)	374	1,220
35	α9	No	**0.496**	11.3 (0.3-418.5)	389	1,248
39	α7	No	0.77	**1.2 (0.8-3.6)**	346	1,169
45	α7	**Yes**	0.71	**0.5 (0.2-1.3)**	373	1,189
51	**α5**	No	0.746	1.9 (1.1-4.0)	327	1,158
52	α9	**Yes**	0.769	1.7 (1.0-3.2)	356	1,077
56	**α6**	No	0.771	2.3 (1.1-4.2)	373	1,151
58	α9	No	0.878	**1.3 (0.8-3.1)**	369	1,193
59	α7	No	**0.476**	-[2]	348	1,224
68	α7	No	**0.284**	2.9 (0.5-16.8)	384	1,232

1: Odds ratio comparing odds of HPV infection in the pre-vaccination periods vs. the post-vaccination period, adjusted for chlamydia positivity, testing venue, age and the assay change between the two periods. Includes data up to end-December 2016

2: does not converge

Table 6.10: Minimum detectable odds ratio for given sample sizes and power

Power (%)	Sample size	HPV58 type-specific prevalence among HPV16/18 negative specimens	Minimum detectable odds ratio
80	Pre-vaccination; n=369	0.025	2.87
		0.05	2.23
	Post-vaccination; n=1,193	0.025	1.89
		0.05	1.61
85	Pre-vaccination; n=369	0.025	3.04
		0.05	2.34
	Post-vaccination; n=1,193	0.025	1.97
		0.05	1.66
90	Pre-vaccination; n=369	0.025	3.28
		0.05	2.48
	Post-vaccination; n=1,193	0.025	2.06
		0.05	1.72

Chapter 7: Estimation of HPV vaccination coverage using serosurveillance (Methods)

7.1. Introduction

I describe the immune response to HPV vaccination in the Background (Section 2.6.1). In summary, results from clinical trials have shown that almost all vaccinated women seroconvert for the HPV types included in the vaccine with, on average, far higher antibody concentrations than those attained following a natural HPV infection. Therefore, serological surveillance of HPV type-specific antibody concentrations in vaccine-eligible populations allows estimation of the proportion of women with a vaccine-induced antibody response. This is useful to confirm HPV vaccination coverage in England but also has the potential to provide additional information not available from the nationally reported data. In particular, research question 4 of this thesis was to compare HPV vaccination coverage among different subgroups of the population and to investigate evidence for potential declines in antibody levels over time since vaccination (i.e. antibody waning). In this chapter I further describe the data sources and methods used to determine vaccine-induced seropositivity for this serosurveillance.

7.2. Rationale of serological surveillance

7.2.1. Why is it important to confirm national HPV vaccination coverage?

Maintaining high HPV vaccination coverage in the population is essential for the success of the national programme. As a consequence, HPV vaccination coverage is one of the Public Health Outcome Framework (PHOF) indicators; these are a series of key public health indicators that allows comparison between counties, districts and regions. Accurate monitoring of this uptake is also important when measuring the impact of the National HPV Immunisation Programme as this will be a predictor for the likely declines in HPV infection and related disease in the longer term. Independent validation of this uptake is of particular interest for the older

vaccination cohorts who were largely vaccinated outside of school as the recording of vaccinations given as ‘mop-up’ doses (i.e. doses given after the initial year that the vaccine was offered) was expected to be less reliable. Full details of how national HPV vaccination coverage is collated by PHE using data collected at the local area level are provided in Section 2.7.2. As discussed, the collection of these data is reliant on manual data recording, often from multiple data sources for each local area.

7.2.2. Why is it important to monitor inequalities in HPV vaccination coverage?

PHE’s key role is “to protect and improve the nation's health and wellbeing, and reduce health inequalities”. There may be inequalities in HPV vaccination coverage; for example, there may be lower vaccination uptake in certain population subgroups leading to a higher subsequent risk of cervical cancer in these women. This is of particular importance if there is a ‘double inequality’, i.e. that women who do not receive HPV vaccination are (i) those at higher risk of HPV infection and/or (ii) those less likely to attend for cervical screening. It is important to be aware of any such inequalities to inform interventions to improve uptake in subgroups with lower coverage.

Furthermore, variations in vaccination coverage in different population subgroups could affect the predicted reductions in cervical cancer due to vaccination, which are based on modelling estimates which do not take inequalities in vaccine uptake or inequalities in attendance at cervical screening into account. If the impact of HPV vaccination on the incidence of cervical cancer is lower than expected, it will be necessary to interpret the reasons for this correctly (i.e. inequalities in vaccination uptake vs. reduced effectiveness of the vaccine) to inform any changes to improve the vaccination programme.

Details of other studies that have examined HPV vaccination uptake in population subgroups in areas of England are provided in Table 7.1[82, 141, 144-146]. In summary, these papers fairly consistently demonstrated lower vaccination coverage among ethnic minorities and also some evidence of lower coverage in those from a lower socio-economic status. However, there were limitations with study design for these studies. One study provided data from an ecological analysis[82] and whilst there was little evidence of associations at the area-level, there may have been associations at the individual level which were not seen using this analysis. Three of the studies[144-146] were limited to certain geographical areas so the results were not necessarily generalisable to the whole population. Furthermore, all five studies included in Table 7.1 relied on either self-collected vaccination status or CHIS records. I have already described potential inaccuracies with CHIS data (Section 5.1.1). Self-collected vaccination using patient questionnaires could have been subject to recall bias (particularly in older women who were offered vaccination at a younger age). Serological surveillance, as conducted in this PhD, offers a robust method to estimate individual HPV vaccination status which is not subject to measurement error due to recall bias or inaccurate recording of vaccination status.

Table 7.1: Studies considering inequalities in HPV vaccination coverage in the UK

Publication	Year	Geographic area	Participants	Sample size	Study design/ Selection of participants	Data sources	Main findings	Critical evaluation
Fisher et al[146]	2008-2011	3 PCTs in South West England	Young females eligible for vaccination who attended school or were resident in the local authority	14,282	Retrospective cohort study. Females were identified through PCT records.	*Demographics* Date of birth, postcode, ethnicity, MMR vaccination details and name of school were obtained from CHIS. Postcode was used to ascertain LSOA deprivation quintile. *Vaccination status* Dates and location of HPV vaccination were obtained from CHIS	*Deprivation* Little evidence of differences in vaccine uptake by quintile of deprivation *Ethnicity* Strong evidence of lower 3-dose uptake among women identifying as black (70%), Asian (75%) or Chinese (71%) compared to White British women (85%)	◦ Missing ethnicity data for 17% women ◦ Potential data coding errors and missing data for vaccination status in CHIS ◦ Limited to one geographical area hence not necessarily representative of general population
Sacks et al[141]	2011	19 hospital-based and 13 community-based SHS across England	13-19 year old females attending sexual health services (SHS) across England	2,247	Cross-sectional study. Eligible females attending SHS were asked by receptionist to complete and return a questionnaire.	*Demographics* Self-completed paper questionnaire on patient demographics and HPV-related risk factors *Vaccination status* Self-completed questionnaire	Lower completion in those offered vaccination (65%) compared to national data. Among those offered the vaccine, there was lower 3-dose completion among black women (55%); women not in education, employment or training (48%); women within London (58%); smokers (59%); and those with an STI diagnosis (53%).	◦ 2247/2861 (79%) completed questionnaires returned ◦ Self-reported HPV vaccination status via questionnaire hence subject to recall bias.

Hughes et al[82]	2008-2011	Data from 151 Primary Care Trusts (PCTs) in England	National vaccination coverage for females offered the HPV vaccine between 2008 and 2011	Area-level data for 151 PCTs	Ecological study.	*Demographics* Area-level deprivation from rank of IMD score for each PCT *Vaccination status* Area-level data from published national HPV vaccination coverage	Some evidence of lower vaccination coverage in more deprived areas for the catch-up cohorts only (Spearman's rank correlation coefficient = 0.10 (p=0.09) for 14-15 yrs and <0.0001 (p<0.0001) for 16-17 yrs.	◦ Vaccination uptake and deprivation are recorded at PCT area-level only. ◦ Other potential risk factors not considered (e.g. ethnicity, religion, sexual behaviour)
Bowyer et al[144]	2012	12 state-funded schools across London	Females in UK school year 11	1,912	Cross-sectional study. All eligible females at schools were given an information sheet and questionnaire (parents of girls could opt the girls out prior to study).	*Demographics* Self-reported data collected on age, ethnicity, religion, household wealth (measured using the Family Affluence Scale), smoking status, sexual behaviour and intention to attend for cervical screening. *Vaccination status* Self-reported	3-dose uptake was lower among girls from black, Asian and "other" ethnic background compared to white girls (85% for white girls; 78% for Asian girls; 69% for black girls; 74% for "other" ethnicity).	◦ 2165/2183 (99%) questionnaires completed. 253 removed due to anomalous results (n=3) or missing vaccination status (n=250). ◦ Self-reported HPV vaccination status via questionnaire hence subject to recall bias. ◦ Limited to a geographical area hence not necessarily representative of general population
Roberts et al[145]	2007-2008	Secondary school attenders at 2 PCTs in Manchester	12-13 year old females	2,817	Prospective cohort study. All females invited for vaccination at relevant PCTs were included in this analysis.	*Demographics* Postcode (obtained from PCT) was used to ascertain LSOA deprivation quintile. Ethnicity was obtained from ethnic monitoring forms sent to participants. *Vaccination status* HPV vaccination data were obtained from CHIS for the relevant PCTs	Uptake was lower in more deprived areas (OR for a 10-point increase in IMD = 0.89; 95% CI 0.85-0.94) and in ethnic minority girls (OR comparing which with non-white ethnicity = 0.67; 95% CI 0.49-0.92).	◦ Ethnicity was not available from CHIS at relevant PCTs hence was requested using forms sent to parents. Only 62% of forms were returned. ◦ These data were from a feasibility study conducted in a specific area prior to the roll out of the National HPV Immunisation Programme. Therefore, these results may not be generalisable.

Abbreviations: CHIS = Child Health Information System; IMD = index of multiple deprivation; LSOA = Lower Layer Super Output Area; OR = Odds ratio; PCT = Primary Care Trust; SHS = Sexual Health Services

7.2.3. Waning of HPV antibodies in vaccinated populations

Data from clinical trials have shown the vast majority of vaccinated women remain seropositive for up to 10 years after vaccination with the bivalent vaccine, and that geometric mean titres remained far higher than those elicited following natural infection[61]. There is no established correlate of protection for the HPV vaccines. It has been suggested that the level of antibodies elicited by these vaccines will likely provide long-term protection[147]. However, the actual duration of protection provided by the current HPV vaccines can be better determined with post-vaccination follow-up of vaccinated women.

7.2.4. Data sources

To investigate the above, I made use of two data sources which I describe in more detail in Sections 7.3 and 7.4:

(i) Sera samples from young women attending for routine microbiological and/or biochemical tests (PHE Sero-epidemiology Unit (SEU)). This serological surveillance of HPV seropositivity was established prior to this PhD (although no analyses to monitor HPV vaccination uptake had been conducted using these data prior to the PhD). The methods of this surveillance are described in Section 7.3. This SEU serosurveillance was designed to confirm national HPV vaccination coverage among young women and to determine if there was any evidence of waning antibody levels among vaccinated women. There was relatively limited patient information collected with these specimens, hence this surveillance did not allow comparison of HPV vaccination coverage in different subgroups of the population.

(ii) Sera samples from young women attending sexual health clinics and having an HIV and/or syphilis test. I established this surveillance as part

of this PhD, as described in Section 7.4. The data from this serosurveillance provides an estimate of the vaccination coverage among women attending sexual health clinics (i.e. those likely to be at higher risk of STIs including HPV infection). Patient data collected as part of this serosurveillance enabled stratification of coverage by demographics to help identify subgroups of the population with lower vaccination coverage. Finally, I designed and conducted a case-control study nested within this surveillance to investigate whether the bivalent vaccine offers protection against genital warts; this is explored further in Section 7.5.

7.3. Methods for SEU serosurveillance

7.3.1. Background

The PHE Sero-Epidemiology Unit (SEU) is part of the Serum Archive Unit and collects residual serum for use in several serological surveillance studies[148]. Participating laboratories are asked to send aliquots of residual serum from routine microbiological and/or biochemical tests. These aliquots are sent to the Vaccine Evaluation Unit (VEU) at PHE along with patient data on age at collection, gender, year of collection and whether the specimen was collected at a sexual health clinic. Specimens from immunocompromised patients and repeat specimens from the same individuals are excluded.

In 2010 (prior to the start of this PhD), it was proposed that HPV serological surveillance could be conducted using these residual SEU specimens to monitor the HPV vaccine-type antibody levels following the introduction of the National HPV Immunisation Programme. For the purposes of this HPV surveillance, the numbers of residual serum specimens requested from women aged 15-19 years old in 2010 to 2013 (i.e. those that would have been eligible to have received the HPV

vaccination as part of the national programme) were increased to approximately 1,000 specimens per year.

7.3.2. HPV antibody testing

Sera specimens were analysed for Immunoglobulin G (IgG) antibodies to HPV16 and HPV18 using a type-specific virus like particle (VLP)-based enzyme linked immunosorbent assay (ELISA). All reagents for the assay, including VLPs, were provided by GSK. Testing was performed at the PHE VEU in Manchester according to the manufacturer's protocols. Briefly, microtiter plates coated with either purified VLP16 or VLP18 antigens were incubated with serial dilutions of sera. The bound antibody was reacted using horseradish peroxidase conjugated goat anti-human IgG and optical density was determined. Quantitative results, expressed as ELISA units per millilitre (EU/mL), were calculated from the standard. Seropositivity was determined using the cut-offs of 19 and 18 EU/mL for HPV16 and HPV18, respectively[149]. Further details of the testing methods are provided in the Methods of the published paper provided in Section 8.2.

7.3.3. Determination of HPV vaccination status

To determine HPV vaccination status of women using the results of anti-HPV16 and 18 antibody testing, I made use of three pieces of prior knowledge, as follows:

(i) Data from clinical trials show that 100% (or close to 100%) of vaccinated women seroconvert for both HPV16 and HPV18 and this remains high for up to 10 years[61].

(ii) Data from clinical trials also demonstrate that, on average, antibody responses in vaccinated women are far higher than antibody responses following a natural HPV infection[61].

(iii) Prior to the introduction of HPV vaccination in England, although 11.7% of 15-20 year old women were seropositive for either HPV16 or HPV18, only

1.8% (95% confidence internal: 0.9%-3.4%) were seropositive for both types (unpublished additional analysis from a previous surveillance study conducted by PHE among 500 females obtained from the PHE SEU prior to the introduction of the National HPV Immunisation Programme[23]).

Using (i) above, I categorised all women who were seronegative for either one or both HPV types as unvaccinated. In those who were seropositive for both HPV types, I wanted to distinguish between vaccinated and unvaccinated women. The above data suggest that women with high antibody concentrations to both HPV16 and HPV18 are likely to have been vaccinated. However, up to 3.4% of women could have a serological response following natural infection (point (iii) above) and whilst average antibody levels following vaccination are higher than those following natural infection, the range of antibody concentrations in these two groups overlap. Therefore, it is not always clear whether those with low antibody concentrations are unvaccinated (having seroconverted after natural infection to HPV16 and HPV18) or vaccinated with a lower than average immune response following vaccination. Similarly, those with higher antibody concentrations are likely to have been vaccinated but could include a small proportion who are unvaccinated but had a higher than average immune response following natural infection.

To determine which women were likely to have been vaccinated, I first used the above knowledge to categorise antibody concentrations for HPV16 and HPV18 as low, medium or high (Table 7.2). As I knew that the majority of women seropositive for both HPV types would have been vaccinated (see point (iii) above), I considered low antibody concentrations as those which were below the 5% range of concentrations among those who were seropositive for both HPV16 and HPV18 (i.e. an unusually low concentration for dual seropositivity, hence presumed largely unvaccinated). Similarly, as I knew that the vast majority (if not all) women seropositive for only one HPV type were unvaccinated, I considered high antibody

concentrations to be above the 95% range of concentrations among those who were seropositive for only one type (i.e. an unusually high concentration for those presumed to have a natural infection). Medium concentrations fell between these two values (Table 7.2).

I then categorised specimens as either:

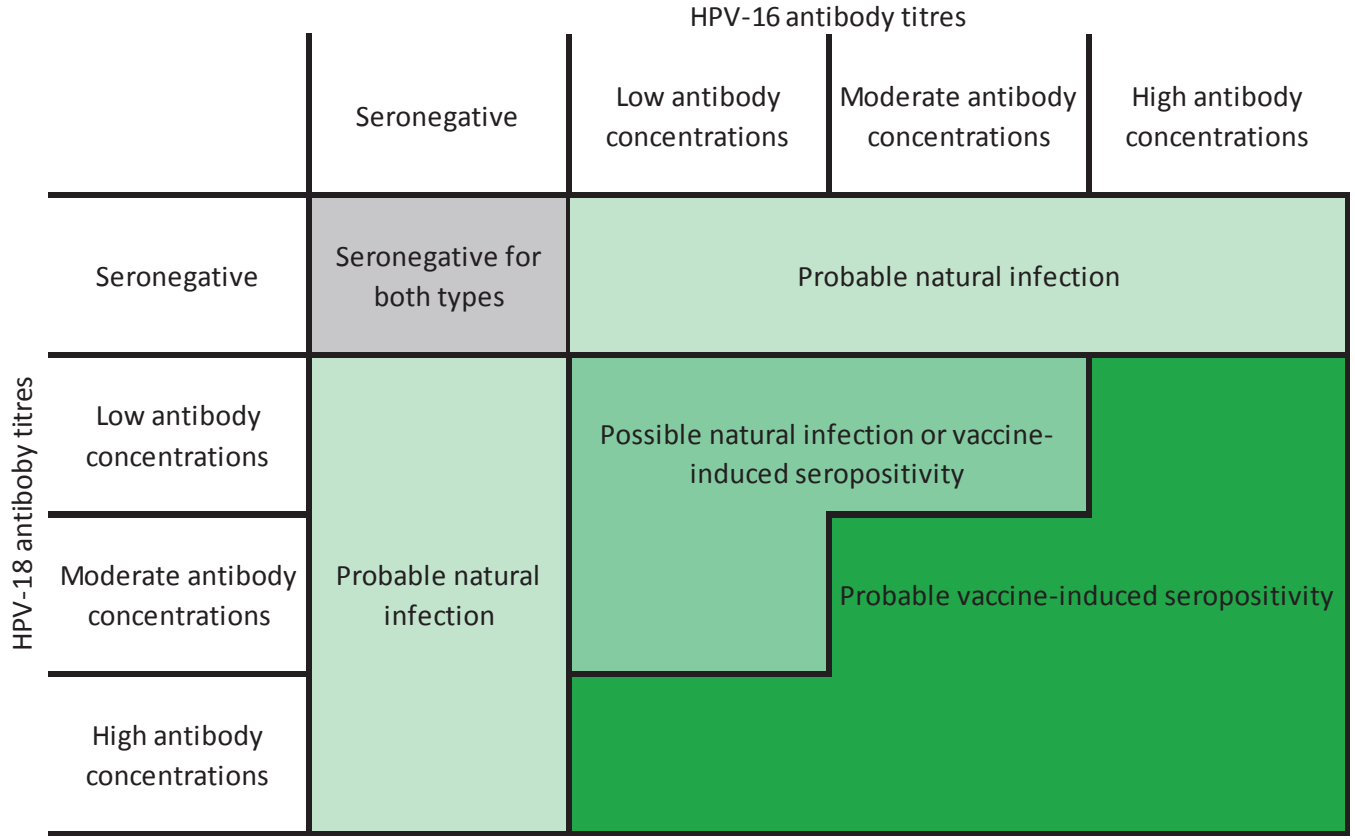
(i) ”Seronegative for both types”: below the assay cut-off for both HPV types.
(ii) “Probable natural infection”: Seropositive for only one HPV type.
(iii) “Possible natural infection or vaccine-induced seropositivity”: Seropositive for both HPV types but with either low antibody concentrations for both HPV types or low concentrations for one type and moderate concentrations for the other.
(iv) “Probable vaccine-induced seropositivity”: Seropositive for both HPV types with moderate antibody concentrations for both HPV types or high antibody concentrations for one or both types.

This is shown graphically in Figure 7.1.

Table 7.2: Classification of HPV16 or HPV18 seropositives

Classification		Description
Seronegative		Antibody concentration below assay cut-off for seropositivity
Seropositive	Low antibody concentrations	Antibody concentration above assay cut-off but below the 5% range of concentrations among those seropositive for both HPV types
	Moderate antibody concentrations	Antibody concentration above the 5% range of concentrations among those seropositive for both HPV types but below the 95% range of concentrations among those with a single antibody
	High antibody concentrations	Antibody concentration above the 95% range of concentrations among those seropositive for only one HPV type

Figure 7.1: Estimation of HPV vaccination status using HPV16 and HPV18 antibody titres



7.3.4. Data analysis

The first HPV vaccine dose is usually given in September (i.e. the start of the academic year). Females included in this SEU surveillance would have been offered 3-doses of the HPV vaccine with the 3rd dose given up to 6 months after the first dose[121]. Therefore, a woman may not be fully vaccinated until March of the following year at the earliest. Consequently, sera which were collected before March of the year following the expected date of a woman's first vaccine dose were excluded in order to monitor seroprevalence following the receipt of the full vaccine course.

As described in the Background (Section 2.7.2), reported HPV vaccination coverage data are published annually for each academic birth year (September to August)[78]. I compared the serological coverage estimates with this published national HPV vaccination coverage. Serological coverage estimates were calculated using two definitions; (i) women with probable vaccine-induced seropositivity, and (ii) women with probable or possible vaccine-induced seropositivity (Figure 7.1).

Age (in years) at the time the serum specimen was taken was known for all women. However, only a proportion of women (~62%) had a known date of birth which meant that the birth cohort (i.e. year that the HPV vaccine would have been offered) was not known. For women with a date of birth, I generated the age and year that the HPV vaccine would have been offered as part of the National HPV Immunisation Programme. For women with no known date of birth, I adapted a previous approach to estimate national vaccination coverage for a calendar year (rather than academic year) for each age-group[84]. For example, a woman aged 17 in 2011 could have a date of birth which falls somewhere over a 24 month period, with the oldest possible woman attending on 1st January 2011 and turning 18 on 2nd January 2011 (i.e. born 2nd January 1993), and the youngest possible woman turning 17 on the 30th December 2011 and attending on 31st December 2011 (i.e. born 30th December

1994). Therefore, a 17 year old in 2011 could fall within one of three different academic birth cohorts (depending on her exact age and the date the sample was taken). Each of these birth cohorts had different national vaccination coverage (based on data reported from the local area) hence I assumed stable attendance patterns through the year to calculate a weighted average for national coverage by age and year, as follows:

$$\text{Coverage} = (m_1/24)*c_1 + (m_2/24)*c_2 + (m_3/24)*c_3$$

Where;

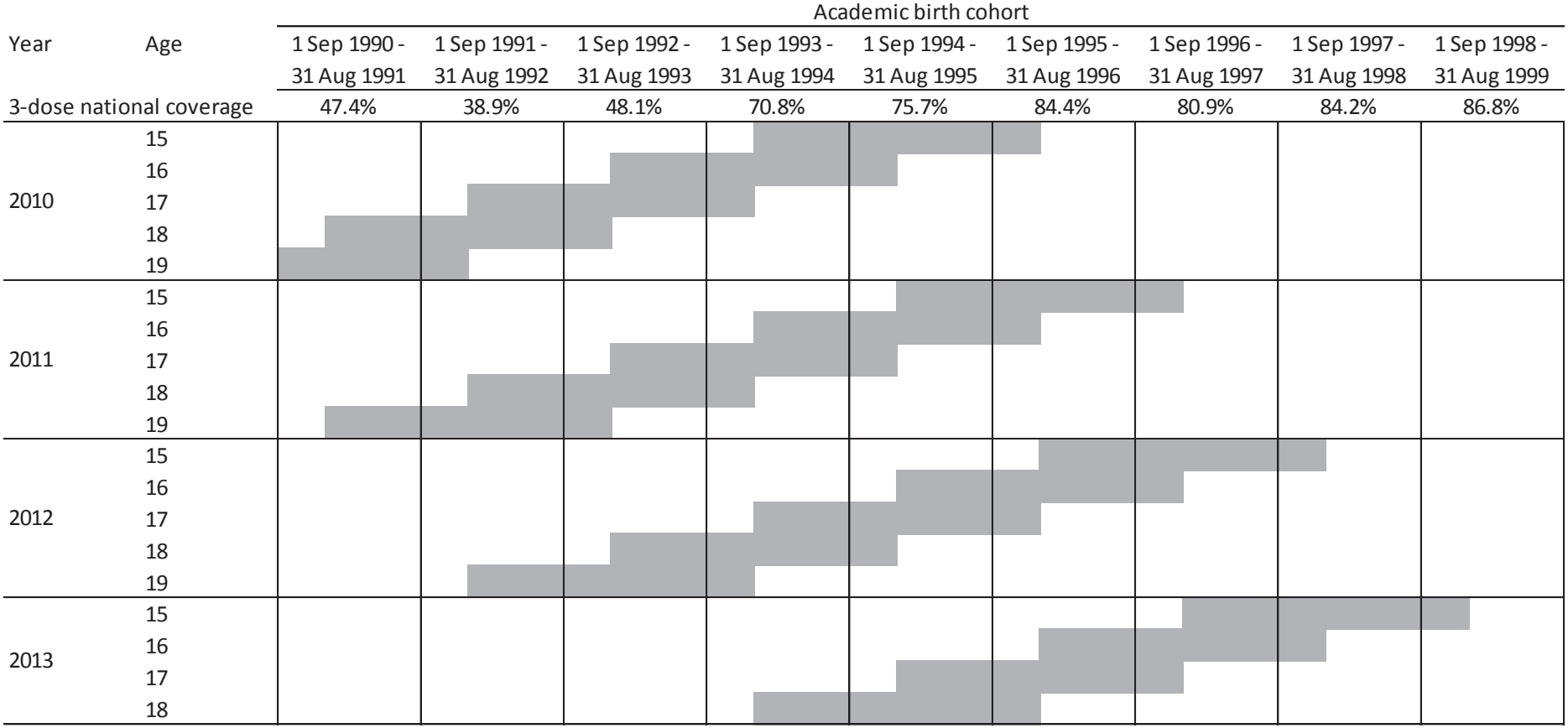
m_i is the number of months in birth cohort i

c_i is the national reported HPV vaccination coverage (as reported by local areas)

In the example above, those born between 2nd January 1993 and 31st August 1993 fall in one birth cohort (with reported national coverage of 48.1%). Those born between 1st September 1993 and 31st August 1994 fall in second birth cohort (with reported national coverage of 70.8%). Those born between 1st September 1994 and 31st December 1994 fall in a third birth cohort (with reported national coverage of 75.7%). Therefore, the estimated national coverage for a 17 year old in 2011 is: (8/24)*48.1% + (12/24)*70.8% + (4/24)*75.7% = 64.1%. This is represented graphically in Figure 7.2.

Antibody concentrations for HPV16 and HPV18 were presented as geometric mean concentrations (GMCs) of EU/mL among seropositive specimens. To explore waning of antibodies since vaccination, I calculated GMCs (with 95% confidence intervals) by time since vaccination was offered (0-1 years, 1-2 years, 2-3 years, 3-4 years and 4-5 years). In this waning analysis, I restricted to women with a known date of birth. Data were plotted, stratified by the age at which the vaccine was offered.

Figure 7.2: Estimation of HPV vaccination coverage by calendar year and age, based on published national coverage



Published 3-dose national coverage is calculated using reported data from local areas

7.4. Methods for serosurveillance among sexual health clinic attenders

7.4.1. Background

The GUMCAD STI surveillance system is a dataset which collects pseudo-anonymised patient-level data on all attendances and diagnoses at sexual health clinics in England. Data collection for GUMCAD commenced on 1st January 2008. An anonymous unique patient ID allows attendances from the same patient to be linked to previous attendances since 2008. In contrast to the SEU data, patient demographics are collected at each attendance, including age at attendance, gender, country of birth, ethnicity and index of multiple deprivation (based on lower layer super output area (LSOA)). Further details are provided in Section 7.4.3.

Surveillance to monitor HPV serology in a high-risk population of young women attending sexual health clinics was established and conducted by me as part of this PhD. The objectives for the serosurveillance among sexual health clinic attenders were to:

(i) Estimate vaccine-induced seroprevalence.

(ii) Compare vaccine-induced seroprevalence to the published national vaccination coverage.

(iii) Compare differences in vaccine-induced seroprevalence between subgroups of the population (ethnicity [categorised as white, black, Asian, other]; quintile of deprivation; whether a patient has a current or previous STI).

Residual sera from females attending a sexual health clinic were requested retrospectively from five laboratories in England which I recruited using the following criteria:

(i) To provide a geographical spread across the country

(ii) To include specimens from women tested in urban and rural areas

(iii) To include laboratories with sufficient numbers of HIV/syphilis blood samples taken (see Section 7.4.2)

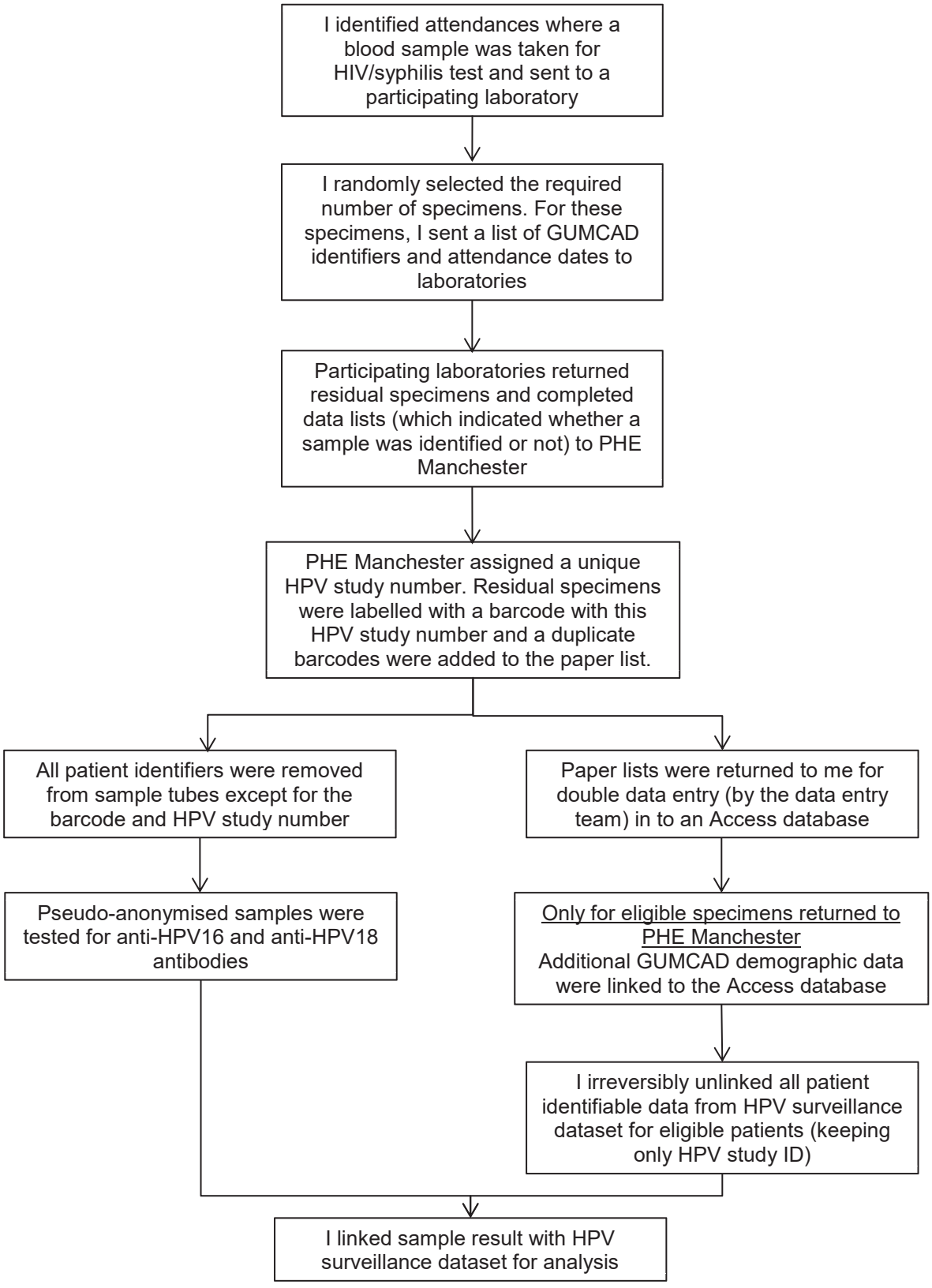
(iv) To be a laboratory that held residual sera samples for at least 1 year (preferably 2 years) to allow for samples to be requested retrospectively

(v) *Optional:* Ideally, to be a laboratory that had been involved with previous PHE surveillance activities

These five laboratories tested specimens collected from six sexual health clinics; Nottingham University Hospital Department of Microbiology (specimens from Nottingham City Hospital GUM clinic); Sheffield Teaching Hospital Microbiology Department (specimens from Royal Hallamshire Hospital sexual health clinic); Homerton University Hospital Department of Microbiology (specimens from Homerton Hospital sexual health clinic); The Countess of Chester Hospital (specimens from Countess of Chester Hospital sexual health clinic); and, Gloucestershire Royal Hospital Microbiology Department (specimens from Cheltenham General Hospital sexual health clinic and Gloucester Royal Hospital GUM clinic).

I selected residual serum specimens from women aged 16-20 years who attended one of these six sexual health clinics between 1st January 2011 and 31st December 2015 and who had a blood sample taken for an HIV and/or syphilis test (identified using GUMCAD data). The process of specimen and data collection are shown in Figure 7.3 (and described in further detail in the next sections).

Figure 7.3: Methods to request and collect residual sera specimens from sexual health clinics attenders and link with corresponding GUMCAD data



7.4.2. Sample size

I calculated the sample size for objective (iii) (Section 7.4.1) which required the largest sample size; specifically, to detect at least a 7.5% difference in coverage by ethnicity (using white women as the reference group), by whether women had a current or previous STI and by quintile of deprivation (using the least deprived quintile as the reference group). I calculated the required number of women in each subgroup (to compare proportions in the subgroup vs. the reference group) using the below formula for comparing a difference in proportions:

$$n = \frac{(z_\alpha + z_\beta)^2 \, (p_1(1-p_1) + \, p_2(1-p_2))}{(p_2 - \, p_1)^2}$$

Where;

n = Number of women in each group

p_1 = proportion of vaccinated women in subgroup 1

p_2 = proportion of vaccinated women in subgroup 2

Smaller population subgroups were not oversampled for this surveillance, and so the final sample size is likely to have had a similar demographic distribution as the total population of female sexual health clinic attenders. For example, if 402 women were of Asian/mixed/other ethnicity, representing 9% of the population, then the total required sample size would be 4,467 women (3,440 (77%) white ethnicity, 625 (14%) black ethnicity and 402 (9%) Asian/mixed/other ethnicity). Therefore, to allow for unequal group sizes, I calculated the total required sample size (Table 7.3), as:

$$N' = \frac{N\,(r+1)^2}{4r}$$

Where,

N' = total sample size adjusted for unequal group size

N = total sample size

r = ratio of uneven groups

Table 7.3: Sample size calculations for each population subgroup with α=0.05 and 80% power

Population subgroup	Proportion of population[1]	HPV vaccine coverage[2]	Sample size	Total sample size
Ethnicity				
White	77%	55%	Reference	
Black	14%	47.5%	411	2,936
Asian/mixed/other	9%	47.5%	388	4,311
Current/previous STI				
No	79%	55%	Reference	
Yes	21%	47.5%	440	2,095
Quintile of IMD				
Q1 (most deprived)	38%	47.5%	Reference	
Q2	18%	55%	514	2,856
Q3	14%	55%	477	3,407
Q4	14%	55%	477	3,407
Q5 (Least deprived)	16%	55%	495	3,094

1: Proportion of 16-20 year old females attending a sexual health clinic and having an HIV/syphilis test recorded in the GUMCAD STI surveillance system (unpublished analyses, conducted by me)

2: Estimates of HPV vaccination coverage were based on (i) published national HPV vaccination coverage (for ages/years included in this surveillance), and (ii) informed by differences between different subgroups from the published literature (Table 7.1)

The final sample size of 4,311 was taken as the highest value in the final column in Table 7.3. To allow for samples which could not be retrieved at local laboratories and for inadequate test results, I increased this number by 10% to give the total number of samples to be requested of 4,742.

7.4.3. Specimen collection

I used GUMCAD to retrospectively identify eligible patient sexual health attendances that included HIV/syphilis tests at relevant clinics to meet the sample size calculations in the previous section. Limited GUMCAD data for each specimen (restricted to fields which were required to identify eligible specimens for this surveillance and to determine case/control status, as outlined in Section 7.5) were saved in a secure Microsoft Access database which I developed specifically for this serosurveillance study. These data fields are summarised in Table 7.4. I generated lists of the clinic ID, clinic patient ID and date of attendance to send to participating laboratories to request residual specimens. In addition, I designed a laboratory protocol including detailed instructions for laboratories on how to select, label and submit samples to PHE (Appendix G1) which was sent along with these lists. These protocol instructions requested laboratories to send aliquots of between 250µl to 2mL for all residual serum specimens on the list that I provided. If a specimen on this list had an identical clinic ID and clinic patient ID to a specimen at the laboratory but the date of attendance on the list was within 7 days of the date recorded at the laboratory (i.e. not an exact match), then I asked the specimen to be sent with a note of the laboratory's recorded date of attendance on the list. If the specimen was not available then laboratories were asked to record this on the list along with a reason (e.g. no specimen within 7 days of the given date, or the specimen had been discarded).

Table 7.4: GUMCAD data extracted for specimens included in the serosurveillance among sexual health clinic attenders

Field Name	Description	Coding
Data fields directly taken from GUMCAD		
clinic_code	Clinic code	Derived by GUMCAD team
patient_id	ID of patient	Derived by sexual health clinic
age	Age at attendance date in years	Numeric (999 Not known)
gender	Gender / sex	1 Male; 2 Female; 9 Not specified/indeterminate
attendance_date	Date of clinic attendance	dd/mm/yyyy
raw_sti_code	SHHAPT code entered by sexual health clinic	See Appendix G2
clean_sti_code	SHHAPT code amended for data coding errors by GUMCAD	See Appendix G2
Derived data fields (using other data from GUMCAD)		
first_date	Earliest date of attendance in GUMCAD	dd/mm/yyyy
last_date	Last date of attendance in GUMCAD	dd/mm/yyyy
p1a	HIV antibody test at attendance	Binary
s2	HIV antibody test and sexual health screen at attendance	Binary
t3	Chlamydia, gonorrhoea and syphilis test at attendance	Binary
t4	Chlamydia, gonorrhoea, syphilis and HIV test at attendance	Binary
c11a_ever	Ever had a first case of genital warts	Binary
c11b_ever	Ever had recurrent genital warts	Binary
c11c_ever	Ever had re-registered case of genital warts	Binary
case	Case definition for nested case-control study (see Section 7.6)	Binary
control	Control definition for nested case-control study (see Section 7.6)	Binary
select	Sample selected for surveillance study	Binary
requested	Sample requested from local laboratory	Binary
match	Identifier to link matched cases and controls	Numeric

Laboratories were asked to send the residual specimens and lists to PHE VEU at Manchester. On receipt, PHE VEU verified that the number of specimens returned matched the number expected according to the data lists. They also generated a unique HPV study number and attached a barcode for each returned sample to the specimen tube with a duplicate barcode attached to the data list (Figure 7.3). PHE VEU retained only this HPV study number to identify residual specimens. The completed lists were then sent to me at PHE Colindale. For each requested specimen on the list, the Access database was updated with information of whether a specimen had been returned (or a reason for not sending). For returned specimens, the HPV study number and the laboratory date of attendance (if different to GUMCAD) were also entered.

7.4.4. GUMCAD data linkage

As described in the previous section, only limited data were held for specimens requested from the laboratories. This was because the GUMCAD team, who review and approve all projects using GUMCAD data, determined that additional patient demographic data should only be linked for those with a residual serum specimen identified by the participating laboratories. Therefore, for returned specimens only, I oversaw, along with a member of the GUMCAD team, the linkage back to GUMCAD data (using clinic ID and clinic patient ID) to obtain the following data: Age (complete for 99% of patients); country of birth (complete for ~90% of patients); ethnicity (complete for ~95% of patients) and LSOA which was used to determine socio-economic status (complete for ~90% of patients). Additionally, a concurrent or previous diagnosis of syphilis, gonorrhoea or chlamydia was also included as a proxy for sexual behaviour. This is shown in more detail in Table 7.5. Following data linkage with GUMCAD, I irreversibly deleted all patient identifiable data (except for the HPV study number) from the Access database and securely discarded any paper records. I released samples for testing after data were pseudonymised.

Table 7.5: Demographic and sexual behaviour from GUMCAD recorded for each specimen requested and returned for the serosurveillance among sexual health clinic attenders

Field Name	Description	Coding
Data fields directly taken from GUMCAD		
country_birth	Patient's country of birth	ISO country codes
sex_orientation	Sexual orientation/risk	1 heterosexual; 2 homosexual; 3 bisexual; 9 not stated/not known
ethnicity	Ethnicity	*White* A British B Irish C Any other White background *Mixed* D White and Black Caribbean E White and Black African F White and Asian G Any other mixed background *Asian or Asian British* H Indian J Pakistani K Bangladeshi L Any other Asian background *Black or Black British* M Caribbean N African P Any other Black background *Other Ethnic Groups* R Chinese S Any other ethnic group Z not stated 99 Not known

la	Local authority (formerly PCT) of residence	National LA codes
lsoa	Lower layer super output authority of residence	National LSOA codes
pct	Primary Care Trust of residence	National PCT codes
Derived data fields (using other data from GUMCAD)		
a1_ever	Ever had a primary Syphilis diagnosis	Binary
a2_ever	Ever had a secondary Syphilis diagnosis	Binary
a3_ever	Ever had an early latent Syphilis diagnosis	Binary
gonn_ever	Ever had a Herpes diagnosis	Binary
chl_date*	Date(s) of chlamydia diagnoses (SHHAPT codes C4A, C4B, C4C or C4D - see Appendix G2)	dd/mm/yyyy (missing if no chlamydia diagnosis)
c10_ever	Ever had a Herpes diagnosis	Binary
imd_rank	IMD rank from LSOA code	Derived from the English indices of deprivation for 2010
imd_score	IMD score from LSOA code	Derived from the English indices of deprivation for 2010

7.4.5. HPV antibody testing

At the start of this surveillance, it was planned that testing for HPV antibodies would be conducted by VEU using the same approach as that used for the SEU serological surveillance. However, there was a problem with availability of the reagents being provided by GSK for testing. Unfortunately, this could not be resolved hence residual sera specimens were transferred from VEU to the German Cancer Research Center (Deutsches Krebsforschungszentrum; DKFZ) in Heidelberg Germany for testing. The 2mL Eppendorf specimen tubes were sent to DKFZ labelled only with the unique HPV study number and no other patient identifiers. Specimens were sent on dry ice in packaging which conformed to UN 3373 regulations for shipment of risk group B samples.

Sera specimens were tested for antibodies to L1 proteins for HPV6, 11, 16 and 18 using a multiplex serology assay which allowed analysis of antibody responses to several antigens in one reaction[150]. Antigens were expressed as Glutathione S-transferase (GST) fusion proteins and affinity-purified on glutathione-derivatized polystyrene beads (Luminex Corp, Austin, TX, USA). Different antigens were purified on different bead sets as defined by the beads' internal fluorescence. The antigen-loaded bead sets were then mixed and incubated with serum. A Luminex flow cytometer distinguished between the bead sets (and hence the loaded antigen) and quantified the amount of bound serum antibody by a human IgG secondary antibody and Streptavidin-R-phycoerythrin fluorescent reporter conjugate. The output was the median reporter fluorescence intensity (MFI) of at least 100 beads per set per sample. Net MFI were generated by subtracting two background values resulting from a blank (a well containing no serum but antigen-loaded beads and all secondary reagents) as well as from a bead set loaded with GST only. Antigen-specific cut-offs were defined by visual inspection of frequency distribution curves (percentile plots) at the approximate inflection point of the curve to dichotomize

antibody responses as seropositive and seronegative (this approach has been used previously in other settings[151-155]). For the surveillance among sexual health clinic attenders described in this chapter, a cut-off of 100 MFI was used to classify seropositivity for HPV16 L1 antibodies and HPV18 L1 antibodies. As a sensitivity analysis, different cut-offs were considered for seropositivity of 80 MFI and 120 MFI.

7.4.6. Determination of HPV vaccination status

Seropositivity for each HPV type could be ascertained using the methods described in the previous section (Section 7.4.5). However, due to unexpected degradation of the glutathione-derivatized polystyrene beads (which was not apparent until after testing had been completed), the MFI results could not be used to quantify antibody concentrations for the serosurveillance among females attending sexual health clinics. This issue meant that, for sexual health clinic attenders, I was unable to apply the same techniques that I developed for the SEU surveillance to determine vaccine-induced seropositivity (described in Section 7.3.3). Consequently, I classified patients as having vaccine-induced seropositivity if they were seropositive to L1 proteins for both HPV16 and HPV18 at the cut-off of 100 MFI. This approach is likely to have misclassified some patients as having a vaccine-induced response whereas, in fact, they had an immune response to both types following natural infection. I discuss the implications of this in Section 8.5.1.

7.4.7. Data analysis

I calculated the proportion of specimens with a valid serological result who were assumed to have vaccine-induced seropositivity in this analysis (i.e. had an antibody response to HPV16-L1 and HPV18-L1). Although oversampling of specific socio-demographic subgroups was not undertaken, women with a diagnosis of genital warts were oversampled (Section 7.5.2). Thus results were weighted to take account of this over-sampling, in order for these to be representative of the population of women attending sexual health clinics and having an HIV and/or

syphilis test taken; samples from women with a diagnosis of genital warts were given less weight and samples from women with no diagnosis of genital warts were given more weight. Specifically, weights were calculated for each year and age as:

$$\text{weight}_{\text{gw}} = \frac{\text{proportion of all women}^{*}\text{ with a diagnosis of genital warts for age/year}}{\text{Proportion of women in HPV surveillance with a diagnosis of genital warts for age/year}}$$

$$\text{weight}_{\text{no gw}} = \frac{\text{proportion of all women}^{*}\text{ with no diagnosis of genital warts for age/year}}{\text{Proportion of women in HPV surveillance with no diagnosis of genital warts for age/year}}$$

* women attending a sexual health clinic included in this surveillance who had an HIV and/or syphilis test recorded in GUMCAD

The prevalence of vaccine-induced seropositivity, with 95% confidence intervals, was presented alongside the published national HPV vaccination coverage. To compare whether vaccination coverage in this high-risk population was different to the national vaccination coverage, I estimated 1-dose and 3-dose coverage for each age and year using national data and the methods previously described (Section 7.3.4).

Comparison of vaccine-induced seropositivity in different subgroups was conducted using a logistic regression model to calculate odds ratios and associated 95% confidence intervals. The prevalence of vaccine-induced seropositivity was compared for the following subgroups; ethnicity (categorised as white, black, Asian, mixed or other); Quintile of deprivation; whether the patient had a concurrent or previous diagnosis of syphilis, gonorrhoea or chlamydia; country of birth (UK vs. outside of the UK); and age at attendance. Adjusted odds ratios were calculated using a multivariable regression model including all these variables. Year of specimen collection was included *a prior* to adjust for potential confounding as more women from younger birth cohorts, with higher vaccination coverage, would have been eligible for this surveillance in later years. As above, odds ratios were weighted for oversampling of women with a diagnosis of genital warts.

7.5. Methods for the case-control study to assess the effectiveness of the bivalent vaccine against genital warts

7.5.1. Background

Genital warts are the most commonly diagnosed viral sexually transmitted condition in high-income country settings. In England, in 2016 there were 27,342 first episodes of genital warts diagnosed in females at sexual health clinics [50]. Recurrence of genital warts was also relatively common with 20,232 recurrent episodes of genital warts in females in the same year[50]. Whilst not associated with severe morbidity or mortality, genital warts have a major impact on patients' quality of life and cause substantial costs to diagnose and treat[51, 156].

National programmes that vaccinate women with the quadrivalent HPV vaccine have been introduced in many countries worldwide. Early data suggest an early and substantial effect on the incidence of genital warts in these countries. In Australia the quadrivalent vaccine has been offered to all girls aged 12 years old since 2007 with a catch-up programme offering the vaccine free-of-charge to all females up to 26 years of age. Ali *et al* report analyses of national surveillance data which showed declines in the rate of genital warts between 2007 and 2011 of 92.6% in Australian-born females aged under 21 years old and 72.6% in females aged 21-30 years old[157]. These data also suggest declines in Australian-born heterosexual men, with an 82% reduction in the proportion with genital warts (likely due to herd immunity as vaccination of men in Australia was not introduced until 2012). In Denmark the quadrivalent vaccine has been offered free of charge to all 12 year old girls since January 2009 with catch-up vaccination offered to all girls up to age 15 since October 2008. Baandruup et al published data which demonstrated an average annual decline in the incidence of genital warts of 45.3% in Denmark among young women aged 16-17 years[158].

Vaccination with the bivalent vaccine was not initially expected to have any protective effect against the low-risk HPV types that cause genital warts since the low-risk types are not closely related to two vaccine HPV types. However, ecological data from sexual health clinics in England have shown a reduction in genital warts diagnoses amongst 16–19 year old females between 2008 and 2011[84]. Declines were positively associated with estimated vaccination coverage. The same pattern was not seen among older women, or for other STIs. Since the start of this PhD, we have updated these ecological analyses (which are not included as part of this PhD). This updated analysis included data collected up to 2014 and demonstrated a 30.6% decline in the diagnosis of genitals warts among women [159]. There was also evidence of a 25.4% decline in heterosexual men of the same age, but no such declines were seen in MSM, potentially suggesting herd protection from female vaccination with the bivalent vaccine. These analyses were supported by a post-hoc analysis of the PATRICIA trial which demonstrated moderate efficacy for the bivalent vaccine against persistent infection with a number of low-risk HPV types[74]. The authors of this study suggested that a plausible mechanism for the cross-protection against these low-risk HPV types could be due to cross-reactivity at the T-helper cell (CD4 receptor) level.

Only a few countries in the world introduced a national vaccination programme using solely the bivalent vaccine (including the Netherlands, England, Scotland, Wales, and Northern Ireland). Of these, England has the most comprehensive surveillance of genital warts diagnoses with the use of GUMCAD data (Section 7.4.1). In England and in the rest of the UK, the HPV vaccine changed from the bivalent to the quadrivalent vaccine in September 2012. Therefore, early data from women vaccinated in the first years of the National HPV Immunisation Programme in England offer a unique opportunity to assess if there is any effect of the bivalent vaccine against genital warts. Such data are important for two reasons. Firstly, this

could inform potential introduction of HPV vaccination and the choice of vaccine in countries who have not yet introduced a vaccination programme. Secondly, these results could affect cost-effectiveness analyses performed to inform changes to the vaccine used in the national programme in England (i.e. bivalent, quadrivalent or nonavalent).

Whilst the ecological data described above provide a suggestion of a moderate protective effect of the bivalent vaccine against genital warts, these data could be affected by other population changes such as changing sexual behaviour or changes in service provision. These are explored in more detail in the above publications but conclude that the declines seen and the specific age- and sex-patterns are suggestive of a direct protective effect of the bivalent vaccine. I therefore established a matched case-control study to further investigate (using individual-level data) whether bivalent HPV vaccination has an effect on genital warts incidence. The matched case-control study was nested opportunistically within residual specimens collected from sexual health clinics as described previously (Section 7.4). The full methods of this case-control study are described below (the results of the study and discussion of the findings are provided in Chapter 8).

7.5.2. Case definition

I defined cases as females aged 16-20 years attending a sexual health clinic between 2011 and 2015, with a diagnosis of a first attack of genital warts (i.e. assumed to be an incident case), and who also had a syphilis or HIV blood test. I had carefully considered the appropriateness of this case definition, taking into account several issues. Firstly, it has been previously estimated that, of all individuals with genital warts who present to either their GP or at a sexual health clinic, around 2.2% would present to their GP only[156]. Therefore, almost all young women with a first diagnosis of genital warts would be expected to attend a sexual

health clinic. Secondly, cases in this study only included those women who had an HIV/syphilis test at the same attendance as their genital warts diagnosis, which limited the number of cases who were eligible and could have affected the sample size for this study. The British Association for Sexual Health and HIV (BASHH) guidelines recommend screening for other STIs in all women diagnosed with genital warts. Therefore, although this restriction does limit the number of available cases of women aged 16-20 years attending a sexual health clinic with a diagnosis of a first attack of genital warts, a relatively high proportion (approximately 60%) were known to have had an HIV or syphilis test at the same visit (unpublished analysis of GUMCAD dataset which I conducted).

7.5.3. Control definition

I defined controls as females aged 16-20 years attending a sexual health clinic for a syphilis or HIV test between 2011 and 2015, with no current or previous diagnoses of genital warts from 2008 to the date of the syphilis or HIV test. Thus, controls were sampled from the population which gave rise to the cases (i.e. sexual health clinics). I describe the matching of cases and controls in the next section (Section 7.5.4) and the number of controls matched to each case in Section 7.5.5).

7.5.4. Selection of cases and controls (concurrent vs. exclusive sampling)

Cases and controls were matched on laboratory and age (years). Prior to July 2012, controls were selected from those without current or previous genital warts diagnosis attending sexual health clinics in the same year of diagnosis as the relevant case (i.e. also matched on year of sample collection). Once selected, cases and controls were ineligible to be reselected. With this exclusive sampling method (due to the restriction of not allowing controls to be reselected), the analyses were limited to estimating an odds ratio which, given the fact that HPV is relatively common, may not be numerically similar to a rate ratio[160]. After I identified this potential problem, I reviewed the selection of controls in this study. As a

consequence of this review, specimens requested from July 2012 onwards were selected using concurrent sampling (otherwise known as density sampling) as, using this method, the case-control odds ratio would estimate a rate ratio[161]. Specifically for this study, I selected controls attending in the same quarter/year of diagnosis of the relevant case (i.e. controls were matched on laboratory, age (in years) and quarter and year of sample collection). Cases could be selected as controls prior to their first genital warts diagnosis[162] which can lead to inconsistent estimators if previously selected controls are excluded from further sampling[163]. Therefore, controls could also be reselected in subsequent quarters as a control (as well as a case) to avoid this potential bias. As only those with a first attack of genital warts were eligible to be cases, a case could not be reselected on subsequent attendances. The potential implications of the selection of controls are further discussed in Sections 8.4 and 8.5.3.

Due to the change in case and control selection to concurrent sampling, I extended the duration of specimen collection from three years to five years (specimens taken between 2011 and 2015) to meet the target sample size (see next Section).

7.5.5. Sample size

The proportion of controls who were vaccinated was assumed to be 50% in this age-group (largely comprising catch-up vaccination cohorts). Power to detect a vaccine effectiveness of the bivalent vaccine against genital warts of 30% and 35% were considered, hence the proportion of cases vaccinated for these two scenarios would be 45.6% and 41.2% respectively (assuming VE=1-OR).

To calculate the sample size in the case of one matched control per case, I used the following formula[164]:

$$n = \frac{[\frac{Z_\alpha}{2} + z_\beta \sqrt{P\,(1-P)}]^2}{(P - 1/2)^2} \over (p_0 q_1 + p_1 q_0)$$

Where;

n = Number of cases
P = OR/(1+OR)
p_0 = proportion of vaccinated controls
p_1 = proportion of unvaccinated controls
q_0 = proportion of vaccinated cases
q_1 = proportion of unvaccinated cases

To calculate the sample size with multiple matched controls per case, I used the following formula to adjust the above sample size calculation (where *c* is the number of matched controls per case)[164]:

$$n' = \frac{(c+1)n}{2c}$$

Using the above formulae, sample size calculations for the number of cases are shown in Table 7.6 with 5% significance level, with power ranging from 80% to 90% and between 1 and 6 matched controls for each case.

Table 7.6: Sample size calculations for matched case-control study (target numbers represent required number of cases)

Proportion of controls vaccinated	Proportion of controls unvaccinated	Vaccine effectiveness (1-OR)	Proportion of cases vaccinated[1]	Proportion of cases unvaccinated[1]	α^2	β^2	Sample size (number of cases) for case:control ratio					
							1:1	1:2	1:3	1:4	1:5	1:6
0.5	0.5	0.3	0.412	0.588	0.05	0.1	666	500	445	416	400	389
0.5	0.5	0.3	0.412	0.588	0.05	0.15	570	428	380	356	342	333
0.5	0.5	0.3	0.412	0.588	0.05	0.2	499	374	333	312	299	291
0.5	0.5	0.35	0.394	0.606	0.05	0.1	459	344	307	287	275	268
0.5	0.5	0.35	0.394	0.606	0.05	0.15	393	295	263	246	236	229
0.5	0.5	0.35	0.394	0.606	0.05	0.2	344	258	230	215	206	201

1: calculated using assumed proportion of controls vaccinated and vaccine effectiveness

2: z_α=1.96 (two-sided) for α=0.05; z_β=1.28, 1.04, 0.84 (one-sided) for β=0.1, 0.15 and 0.2 respectively

Further to the above, I first added 15% to allow for multivariable regression analysis and then added a further 10% to the above sample size calculations to account for specimens which (i) could not be retrieved at local laboratories, or (ii) had an inadequate serology result (Table 7.7).

I requested a sample size of 420 cases with 3 matched controls for each case. Requesting additional controls for each case would have provided limited additional power. With this number of cases and controls (allowing for those not retrieved or with an inadequate test result), this gives over 90% power to identify a vaccine effectiveness of 35% and around 80% power for a vaccine effectiveness of 30% (Table 7.7).

Table 7.7: Sample size calculations for matched case-control study (target numbers represent required number of cases) allowing for an additional 10% for specimens which could not be retrieved or had an inadequate serology test and 15% for multivariable regression

Proportion of controls vaccinated	Proportion of controls unvaccinated	Vaccine effectiveness (1-OR)	Proportion of cases vaccinated[1]	Proportion of cases unvaccinated[1]	α^2	β^2	Sample size (number of cases) for case:control ratio					
							1:1	1:2	1:3	1:4	1:5	1:6
0.5	0.5	0.3	0.412	0.588	0.05	0.1	842	633	563	526	506	492
0.5	0.5	0.3	0.412	0.588	0.05	0.15	721	541	481	450	433	421
0.5	0.5	0.3	0.412	0.588	0.05	0.2	631	473	421	395	378	368
0.5	0.5	0.35	0.394	0.606	0.05	0.1	581	435	388	363	348	339
0.5	0.5	0.35	0.394	0.606	0.05	0.15	497	373	333	311	299	290
0.5	0.5	0.35	0.394	0.606	0.05	0.2	435	326	291	272	261	254

1: calculated using assumed proportion of controls vaccinated and vaccine effectiveness

2: z_α=1.96 for α=0.05; z_β=1.28, 1.04, 0.84 for β=0.1, 0.15 and 0.2 respectively

7.5.6. Data analysis

I conducted all statistical analyses in Stata v13.

HPV vaccination status was determined as previously described (Section 7.4.6). For the case-control analysis, I performed two separate analyses, as follows:

The first analysis included all cases and controls regardless of how they were selected (i.e. exclusive sampling or concurrent sampling). I included the variables used for the matching (quarter of specimen collection, age and laboratory) in an unconditional logistic regression model[165]. To adjust for other potential confounding between case/control status and vaccination status, I further adjusted for adjusted for ethnicity (white, black and Asian, mixed ethnicity or other ethnicity), country of birth (UK or outside of the UK), quintile of deprivation (calculated using LSOA of residence if available or LSOA of clinic otherwise) and whether the patient had been diagnosed with syphilis, gonorrhoea or chlamydia (either at the time the serum specimen was taken or previously).

The second analysis included only cases and controls selected by concurrent sampling (selected from July 2012 onwards as described in detail in Section 7.5.4). I performed a conditional logistic regression for a matched analysis. Cases and controls that could not be retrieved from local laboratories or that had an inadequate serology result were excluded from the analysis. If a case was excluded then any matched controls were also excluded. If a control was excluded then the case was retained along with remaining controls and the analysis was conducted with a variable number of matched controls per case. If all matched controls for a case were excluded then the case was also excluded. As above, conditional multivariable regression model was adjusted for ethnic group, country of birth, quintile of IMD and presence of an STI in a multivariable regression model.

For both analyses, unadjusted and adjusted odds ratios (which estimated rate ratios for the second analysis) and 95% confidence intervals were presented. There were some missing data for ethnicity, country of birth and quintile of deprivation. For both analyses, I conducted a complete-case analysis including only individuals with no missing information for adjustment variables.

In summary, in this Chapter I have outlined the methods I developed to estimate HPV vaccination status, based on the results of HPV immune responses in residual serum specimens. I applied these methods to two distinct populations. I also outlined methods for a case-control study which I designed to estimate the effect of the bivalent HPV vaccine on the incidence of genital warts. The results of the two surveillance studies and of the case-control study are presented in the next chapter (Chapter 8).

Chapter 8: Results of serological surveillance to estimate HPV vaccination coverage and vaccine effectiveness against genital warts

8.1. Introduction

In this chapter, I give the results of the two separate surveillance activities to monitor HPV vaccination coverage using results from immunological testing of serology specimens. This chapter includes two manuscripts and a report with updated analyses from the first manuscript.

The aim of the analysis included in the first manuscript, published in PLOS One in 2016, was to confirm the reportedly high proportion of women in the population who have received the HPV vaccine (based on aggregate data compiled and published by PHE) by considering the immune response in a sample of women broadly representative of the general population (the SEU serosurveillance). The results reported in the paper were updated with an analysis I carried out with an additional two years of data. These updated results were made available in 2016 as a PHE Health Protection Report; this report is also included in this Chapter.

The second manuscript (*about to be submitted*) explores two quite distinct research questions. Firstly, I present estimated vaccination coverage in a population of young women attending a sexual health clinic for an HIV and/or syphilis test between 2011 and 2015 (the serosurveillance among sexual health clinic attenders). I also compare vaccination coverage in different population subgroups. Secondly, I report the results of the nested case-control study designed to investigate the potential effectiveness of the bivalent vaccine against genital warts. After these papers, I have included some additional discussion about the limitations of the serosurveillance among sexual health clinic attenders and potential biases in the results.

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SECTION A – Student Details

Student	David Mesher
Principal Supervisor	Sara Thomas
Thesis Title	Assessment of the population-level impact of a high coverage HPV immunisation programme in young females

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

Where was the work published?	PLoS One		
When was the work published?	March 2016		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	N/A		
Have you retained the copyright for the work?*	**Yes**	Was the work subject to academic peer review?	**Yes**

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SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	
Please list the paper's authors in the intended authorship order:	
Stage of publication	Choose an item.

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	The Public Health England (PHE) Seroepidemiology Unit (SEU) is a source of residual sera archived for surveillance activities and held at the PHE Vaccine Evaluation Unit, established before the start of my PhD. The initial idea to use residual sera to monitor HPV vaccination uptake was developed by Kate Soldan, Ray Borrow and

	Richard Pebody. Laboratory testing was performed by Elaine Stanford, Jamie Findlow, Rosalind Warrington and Ray Borrow. I designed and conducted the data analysis, including developing techniques to ascertain HPV vaccination status from results of HPV serology testing. I wrote the first draft of this paper which was commented on by all authors. This paper was peer reviewed and I incorporated suggestions from reviewers and responded to their comments, with input from other authors.

Student Signature: [redacted] **Date:** 12/04/2018

Supervisor Signature: [redacted] **Date:** 13/04/18

PLOS ONE

RESEARCH ARTICLE

HPV Serology Testing Confirms High HPV Immunisation Coverage in England

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Competing Interests: The authors of this manuscript have the following competing interests: RB, JF, ES and RW are employees of PHE and undertake contract research on behalf of PHE for pharmaceutical companies, including GlaxoSmithKline, Novartis, Sanofi Pasteur MSD, Baxter and Pfizer. JF has acted as a consultant on behalf of PHE and has received travel assistance

Abstract

Background

Reported human papillomavirus (HPV) vaccination coverage in England is high, particularly in girls offered routine immunisation at age 12 years. Serological surveillance can be used to validate reported coverage and explore variations within it and changes in serological markers over time.

Methods

Residual serum specimens collected from females aged 15–19 years in 2010–2011 were tested for anti-HPV16 and HPV18 IgG by ELISA. Based on these results, females were classified as follows: seronegative, probable natural infection, probable vaccine-induced seropositivity, or possible natural infection/possible vaccine-induced seropositivity. The proportion of females with vaccine-induced seropositivity was compared to the reported vaccination coverage.

Results

Of 2146 specimens tested, 1380 (64%) were seropositive for both types HPV16 and HPV18 and 159 (7.4%) positive for only one HPV type. The IgG concentrations were far higher for those positive for both HPV types than those positive for only one HPV type. 1320 (62%) females were considered to have probable vaccine-induced seropositivity. Among vaccine-induced seropositives, antibody concentrations declined with increasing age at vaccination and increasing time since vaccination.

Conclusions

The proportion of females with vaccine-induced seropositivity was closest to the reported 3-dose coverage in those offered the vaccination at younger ages, with a greater discrepancy in the older females. This suggests either some under-reporting of immunisations of older

from GlaxoSmithKline, Novartis, Baxter and Pfizer. RB is an Academic Editor of PLOS ONE. All other authors have no conflicts of interest. This does not alter the authors' adherence to PLOS ONE policies on sharing data and materials.

females and/or that partial vaccination (i.e. one- or two-doses) has provided high antibody responses in 13–17 year olds.

Introduction

A national HPV immunisation programme was introduced throughout the UK in September 2008 with routine vaccination offered to all girls aged 12–13 years and a catch-up programme in the first two years offering the vaccination to all girls up to the age of 18 years. From 2008 to 2011, the bivalent vaccine was offered with a change in September 2012 to the quadrivalent vaccine. HPV vaccination is offered free of charge to all girls. The immunisation programme is primarily delivered in schools but also in General Practitioners (GPs) and other health care services, particularly for the older catch-up cohorts. Reported vaccination coverage has been high with over 80% of girls in the routine cohorts completing the three dose schedule [1–4]. Reported coverage is based on data provided by local areas, collated and monitored by Public Health England (PHE).

In females, following a natural infection with HPV, a detectable antibody response is only detected around 50–70% of the time [5–8] and this response is usually fairly weak. Vaccination induces seroconversion in close to 100% of recipients and results in substantially higher average IgG concentrations than following natural infection [9].

Accurate, validated knowledge of HPV vaccination coverage is important to assess the likely direct impact of the HPV immunisation programme as well as the potential indirect effect of herd protection among the unvaccinated. Monitoring of serological markers can also enable vigilance for potential lower levels of direct protection from the immunisation programme within certain sub-groups, and for changes in immunogenicity over time, i.e. antibody waning, which may presage reductions in protection.

We have used distributions of anti-HPV 16 and HPV 18 IgG concentrations to classify sera from a sample of young females in England as probable vaccine-induced seropositive or probable natural infection. We compare the resulting estimates of coverage derived from anti-HPV IgG concentrations to reported vaccination coverage, and explore associations between antibody levels and age at vaccination and time since vaccination.

Materials and Methods

Ethics statement

National Research Ethics Service (NRES) approval for the sero-epidemiological surveillance of the National Immunisation programme of England and Wales (Research Ethics Committee number 05/Q0505/45) was granted by the Joint University College London/University College London Hospital (UCL/UCLH) Committees on the Ethics of Human Research.

Patient consent was not required as this study made use of anonymised specimens (with no patient identifiable data) which were collected and tested as part of Public Health Surveillance conducted to monitor the HPV vaccination programme.

Residual serum specimens

Serum specimens from females aged 15–19 years were obtained from the PHE Seroepidemiology Unit (SEU). The SEU routinely collects residual serum specimens after diagnostic microbiological tests for seroepidemiological studies of infections of public health importance for

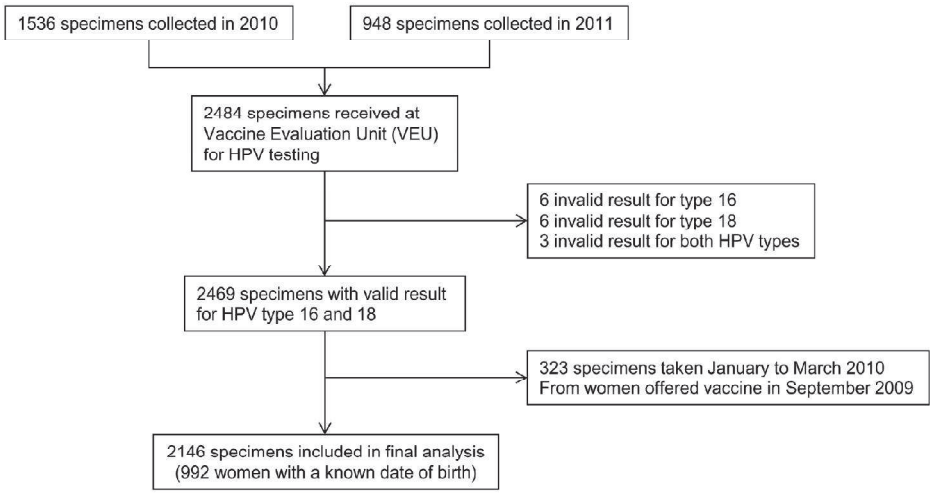

Fig 1. Flow chart of eligible samples.

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which vaccines are available or under development. Contributing laboratories in England provide anonymised specimens with age at collection, sex, and date of collection. Sera from immunocompromised individuals and repeat sera from the same individuals are excluded [10]. Where possible, laboratories identify specimens that originated from Genitourinary Medicine (GUM) clinics. We increased collection of specimens from females aged 15–19 years old (i.e. who would have been eligible to receive the bivalent HPV vaccine as part of the national HPV immunisation programme) by approximately 1000 specimens per year for the purposes of this study. A total of 2484 serum specimens were collected from 12 contributing laboratories between January 2010 and December 2011 (Fig 1). Where exact age at sample collection was available, this was used to generate the age and calendar year that HPV vaccination would have been offered: this was available for 992/2146 (46.2%) of women. For the remainder, with age in years available, likely year of eligibility for HPV vaccination was estimated. Specimens collected in January-March following the due date of first vaccine dose were excluded in order to study seroprevalence after, not during, the scheduled full course of immunisation. Analyses considering time since vaccination and age at vaccination were restricted to women with a known exact age.

HPV testing and serological coverage

Specimens were tested at the PHE Vaccine Evaluation Unit (VEU), Manchester for IgG to HPV types 16 and 18 using a type-specific ELISA and all assay critical reagents, including Virus Like Particles (VLPs), transferred from GlaxoSmithKline [11]. Briefly, VLP16 and VLP18 antigens, purified from recombinant Baculovirus were pre coated onto separate 96 well microtitre plates for between 60 and 120 hours at 4°C. Following blocking to prevent non-specific binding, test, negative control, positive control and standard serum were added to VLP 16 and VLP 18 plates, in serial two-fold dilutions and incubated for 60 minutes at room temperature. Specific bound antibody was detected using horseradish peroxidase goat anti-human IgG

conjugate and developed with a specific chromogenic substrate. Optical density was determined at 450nm with a 620nm reference. Quantitative results were calculated from the standard and expressed in arbitrary ELISA units per millilitre (EU/mL). The lower limit of quantitation of the assay at the VEU was 19 and 18 EU/mL for HPV16 and HPV18, respectively, with values below this classed as seronegative.

Antibody concentrations are presented as geometric mean concentrations (GMCs) among seropositive specimens. Whilst average antibody levels following vaccination are far higher than those following natural infection, the ranges overlap. Using the range of concentrations for types 16 and 18 seropositives we classified each result as, (i) "high" seropositivity if the result was above the 95% range of concentrations among those with a single antibody (i.e. unusually high for presumed largely naturally infected); (ii) "low" seropositivity as below the lower 95% range of concentrations among those seropositive for both HPV types (i.e. unusually low for dual seropositivity, presumed largely immunised); (iii) "moderate" seropositivity as between these two values. Using this grading, we then classified "probable" vaccine-induced seropositivity as seropositive for both types with high concentration for at least one type or moderate concentrations for both types and "probable" natural infection as seropositive for one type only. Specimens with low seropositivity for both types or low seropositivity for one type and moderate for the other were classified as "possible" natural infection or vaccine-induced seropositivity (Fig 2). Serological coverage estimates were calculated as vaccine-induced seropositives divided by the total number of sera with valid test results.

Reported HPV vaccination coverage

Data on reported coverage for each birth cohort included in our seroprevalence data were obtained from published tables [1]. Briefly, annual data on the number of girls receiving at least one, at least two doses or all three doses of the vaccine for each area are submitted to the ImmForm website, a web-based reporting system managed by PHE, using denominators based on the appropriate age-specific school-roll data for females, obtained from the Department for Education.

For comparison with serological coverage, vaccination coverage was estimated using the published coverage for the 10 geographical areas (Strategic Health Authorities) of the laboratories submitting serum specimens, as well as using national level data. Published coverage, reported by academic year (September to August), was used to estimate coverage by age and calendar year. As estimates using Strategic Health Authority data and national data for reported vaccination coverage were similar, the estimates from national data were used.

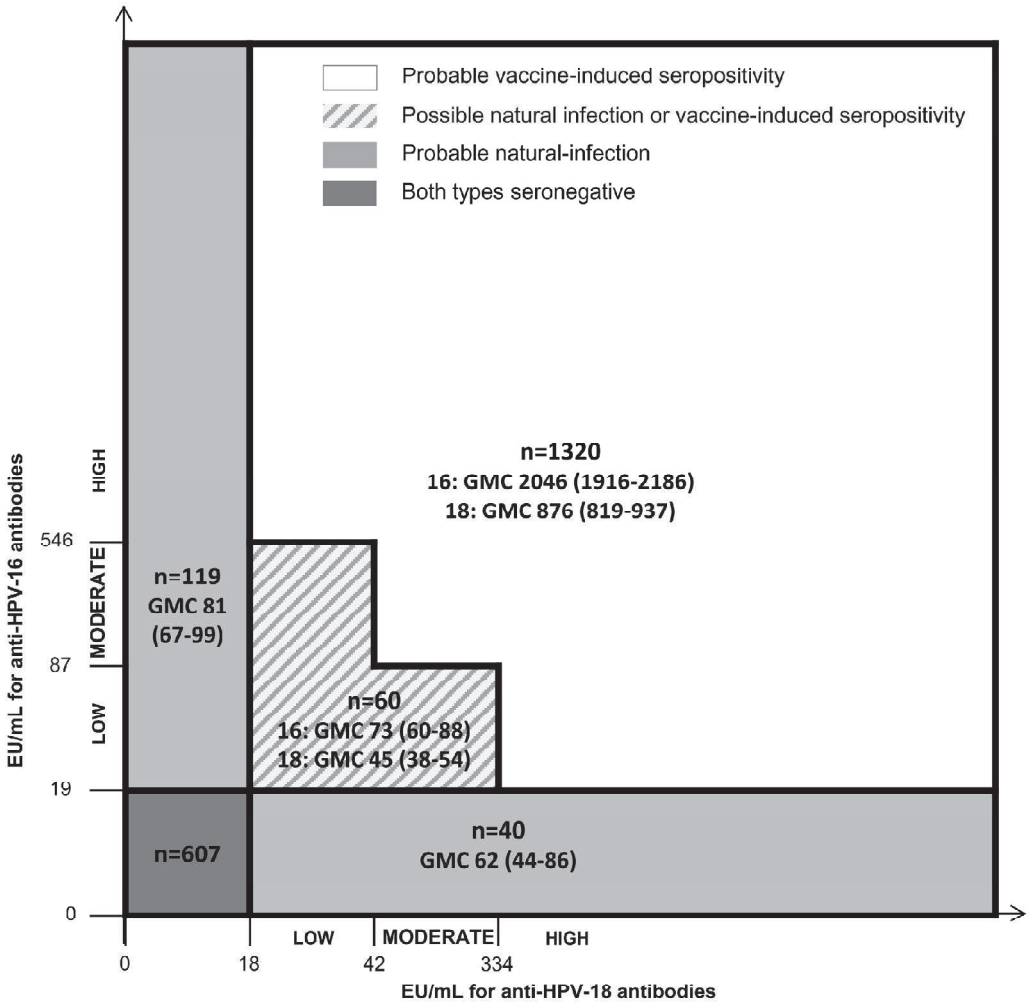
Results

A valid result for both HPV 16 antibodies and HPV 18 antibodies was available for 2469 of the 2484 specimens (99.4%). 323 specimens with date of collection during January to March of the year following the due date of first vaccine dose were excluded: 2146 specimens were included in the analysis (Fig 1).

The mean age of females providing a specimen was 17.9 years (SD 1.4 years). Around one-third of specimens were known to have originated from GUM clinics (ranging from 0% to 93% by contributing laboratory). The mean age was similar for specimens from GUM clinics and those from unspecified source clinics (17.8 years and 17.9 years, respectively).

Seropositivity for vaccine HPV-types

Across all ages, 64% (1380) of specimens were seropositive for both HPV 16 and HPV 18. Seropositivity for HPV 16 only and for HPV 18 only was found in 5.5% (119) and 1.9% (40) of



Using the range of concentrations for types 16 and 18 seropositives we classified each result as, (i) "high" seropositivity if the result was above the 95% range of concentrations among those with a single antibody (i.e. unusually high for presumed largely naturally infected); (ii) "low" seropositivity as below the lower 95% range of concentrations among those seropositive for both HPV types (i.e. unusually low for dual seropositivity, presumed largely immunised); (iii) "moderate" seropositivity as between these two values.

Fig 2. Definition of natural infection and vaccine-induced seropositivity. Abbreviations: EU/mL = ELISA units per millilitre per millilitre; GMC = Geometric mean concentration.

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specimens, respectively. 28% (n = 607) were seronegative for both HPV types (Fig 2). The GMCs were over 10-fold higher for specimens which were seropositive for both HPV types (GMC of 1770 EU/mL for HPV type 16 and 770 EU/mL for HPV type 18) than among those seropositive for only one type (GMC of 81 EU/mL for HPV type 16 and 62 EU/mL for HPV type 18).

Table 1. Seropositivity for HPV16 and 18 by clinical setting and laboratory sending specimens

	Number with valid result n	Proportion seropositive for HPV 16 and/or 18 n (%)	Vaccine-induced seropositivity n (%)	Natural infection seropositivity n (%)
Total	*2,146*	*1,539 (71.7)*	*1,320 (61.5)*	*219 (10.2)*
Clinical setting				
Genito-urinary Medicine (GUM) clinic	798	609 (76.3)	535 (67.0)	74 (9.3)
Unknown clinic setting	1,348	930 (69.0)	785 (58.2)	145 (10.8)
Age specimen taken				
15 years	314	249 (79.3)	236 (75.2)	13 (4.1)
16 years	361	286 (79.2)	263 (72.9)	23 (6.4)
17 years	324	236 (72.8)	200 (61.7)	36 (11.1)
18 years	523	364 (69.6)	303 (57.9)	61 (11.7)
19 years	624	404 (64.7)	318 (51.0)	86 (13.8)
Laboratory[a]				
North East				
Newcastle	290	240 (82.8)	207 (71.4)	33 (11.4)
North West				
Manchester	289	209 (72.3)	183 (63.3)	26 (9.0)
Yorkshire and The Humber				
Leeds	530	378 (71.3)	314 (59.2)	64 (12.1)
East Midlands				
Cambridge	95	61 (64.2)	53 (55.8)	8 (8.4)
Leicester	172	145 (84.3)	138 (80.2)	7 (4.1)
West Midlands				
Birmingham	27	12 (44.4)	9 (33.3)	3 (11.1)
London				
Barts and The London	199	112 (56.3)	93 (46.7)	19 (9.5)
St George's Hospital	105	50 (47.6)	37 (35.2)	13 (12.4)
South Central				
Southampton	9	6 (66.7)	5 (55.6)	1 (11.1)
South East				
Brighton	49	30 (61.2)	24 (49.0)	6 (12.2)
South West				
Bristol	25	22 (88.0)	19 (76.0)	3 (12.0)
Exeter	331	257 (77.6)	222 (67.1)	35 (10.6)
Gloucester	25	17 (68.0)	16 (64.0)	1 (4.0)

[a] p-value for heterogeneity across laboratories; p<0.0001 for proportion seropositive for HPV 16 and/or 18, p<0.0001 for proportion with vaccine-induced seropositivity, p = 0.415 for proportion with natural infection seropositivity.

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Overall seropositivity for HPV16 and/or HPV18 was higher in specimens known from GUM clinics (76.3% vs. 69.0% for HPV 16 and/or 18) and specimens from younger ages (Table 1).

Vaccine-induced seropositivity (VIS)

Within this sample of serum, using the methods described, probable vaccine-induced seropositivity (VIS) was defined as sera with antibody concentrations above 546 EU/mL for HPV 16 or

Table 2. Seropositivity for HPV16 and 18 amongst all specimens tested for both HPV types, by age.

	Age in years					
HPV type	15	16	17	18	19	*Total*
All women	***314***	***361***	***324***	***523***	***624***	***2,146***
Both types negative	21% (65)	21% (75)	27% (88)	30% (159)	35% (220)	*28% (607)*
Natural infection seropositivity:						
- Probable 18 only	1.0% (3)	0.8% (3)	1.9% (6)	2.1% (11)	2.7% (17)	*1.9% (40)*
- Probable 16 only	1.0% (3)	3.3% (12)	6.2% (20)	6.9% (36)	7.7% (48)	*5.5% (119)*
- Probable 16 or 18	1.9% (6)	4.2% (15)	8.0% (26)	9.0% (47)	10.4% (65)	*7.4% (159)*
- Probable and possible	4.1% (13)	6.4% (23)	11.1% (36)	11.7% (61)	13.8% (86)	*10.2% (219)*
Vaccine-induced seropositivity:						
- Probable	75% (236)	73% (263)	62% (200)	58% (303)	51% (318)	*62% (1320)*
- Probable and possible	77% (243)	75% (271)	65% (210)	61% (317)	54% (339)	*64% (1380)*
Expected 1-dose (national)	84.2%	77.7%	66.7%	60.5%	48.9%	-
Expected 2-dose (national)	82.1%	75.0%	63.1%	55.5%	44.1%	-
Expected-3-dose (national)	78.3%	70.1%	55.8%	45.5%	35.1%	-

"Probable" vaccine-induced seropositivity defined as seropositive for both types with high concentration for at least one type or moderate concentrations for both types. "Probable" natural infection as seropositive for one type only. "Possible" natural infection or vaccine-induced seropositivity defined as low seropositivity for both types or low seropositivity for one type and moderate for the other.

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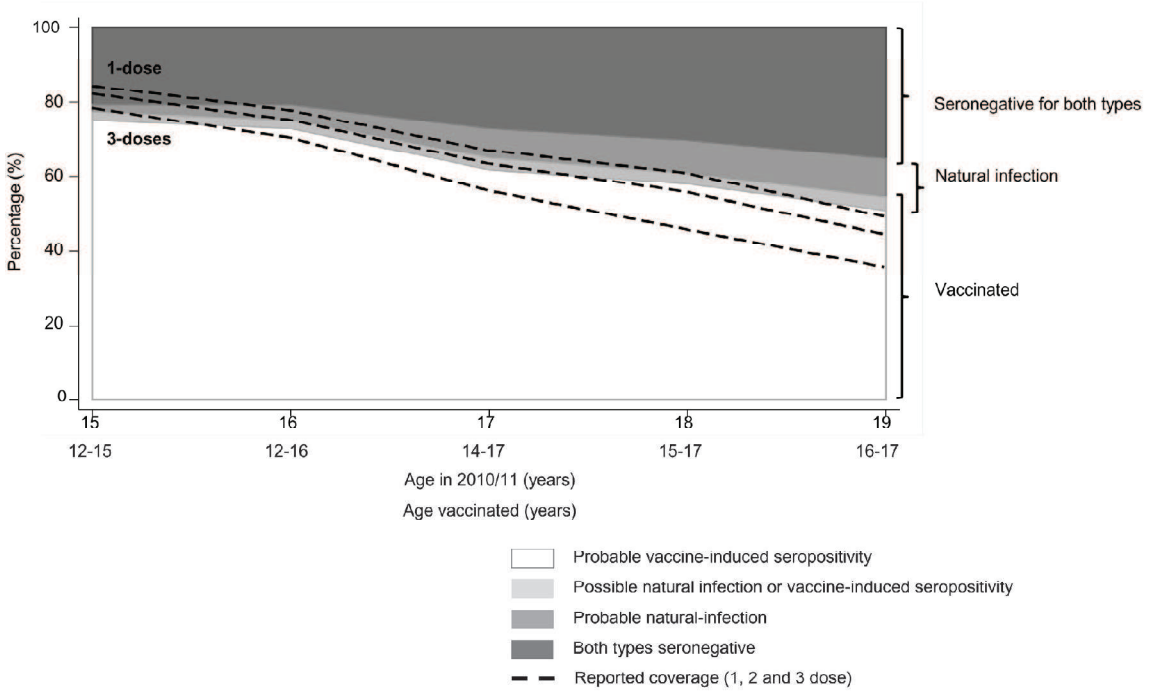
above 334 EU/mL for HPV 18 (and seropositive for HPV 18 and HPV 16, respectively), or above 87 EU/mL for HPV 16 and above 42 EU/mL for HPV 18 (Fig 2). The overall proportion of females with probable VIS was 61.5% (1320) with GMCs of 2046 EU/mL (95%CI 1916–2186) and 876 EU/mL (95%CI 819–937) for HPV 16 and 18 respectively. An additional 2.8% (60) were possible natural infection or possible VIS (with GMCs of 72.9 EU/mL (95%CI 60.1–88.5) for HPV 16 and 45.1 EU/mL (95%CI 37.8–53.8) for HPV 18). The proportion of females with vaccine-induced seropositivity was slightly lower than the reported three-dose coverage for 15 year olds but higher at older ages. There was increasing discrepancy between reported coverage and the proportion of females with vaccine-induced seropositivity with increasing age (Table 2 and Fig 3).

Among the probably VIS, GMCs for HPV16 were higher than GMCs for HPV18 at all ages. For both HPV 16 and HPV 18, GMCs declined with increasing time since vaccination (up to 3-years data available). Specimens estimated to have been taken at equal times after vaccination tended to have higher GMCs if vaccinated at younger ages (at two-years following vaccination, GMCs in 12 year olds were 2561 EU/mL (95%CI 1273–5154) and 1296 EU/mL (95%CI 632–2656) for types HPV16 and HPV18 respectively, whereas in 14–17 year olds these were lower at 1631 EU/mL (95%CI 1422–1871) and 669 EU/mL (95%CI 581–770) respectively) (Fig 4).

Natural infection seropositivity

The GMCs for those with probable natural infection (i.e. seropositive for only one HPV type) were 81.1 EU/mL (95%CI 66.6–98.7) for HPV 16 and 61.7 EU/mL (95%CI 44.2–86.0) for HPV 18. GMCs for those seropositive for both types but with possible natural infection were similar (72.9 EU/mL (60.1–88.5) and 45.1 EU/mL (37.8–53.8) for HPV 16 and 18, respectively). The proportion of females with probable or possible natural infection was similar in specimens known to be submitted from GUM clinics (9.3% vs. 10.8% for those from a GUM clinics vs.



"Probable" vaccine-induced seropositivity defined as seropositive for both types with high concentration for at least one type or moderate concentrations for both types. "Probable" natural infection as seropositive for one type only. "Possible" natural infection or vaccine-induced seropositivity defined as low seropositivity for both types or low seropositivity for one type and moderate for the other

Fig 3. Published HPV vaccine coverage and vaccine induced seropositivity by age (n = 2,146).

doi:10.1371/journal.pone.0150107.g003

those from an unspecified clinic, respectively). The probable and possible natural infections increased with increasing age (Fig 3).

Discussion

Serological surveillance confirms high coverage of the HPV vaccination programme in young females in England, particularly in those offered the vaccine at school age. The higher proportion with vaccine-induced seropositivity compared to reported three-dose coverage, particularly evident in the older females (offered HPV vaccination at an older age), suggests that three-dose coverage in the catch-up cohorts could be higher than reported, or that two-dose coverage at these ages is associated with high antibody responses, or both.

We used the results from serological testing to determine the vaccination status of females. Previous studies have shown only a relatively small proportion of females have natural seropositivity for both HPV types 16 and 18 [12]. Conversely, data from clinical trials show close to 100% of vaccinated females seroconvert for both HPV types [13] with no substantial waning of seropositivity up to seven years following vaccination [14]. Whilst antibody levels are generally far higher in vaccinated females [14], the antibody levels required to protect against HPV infection are unknown and there is an overlap in the ranges of concentrations in vaccinated and

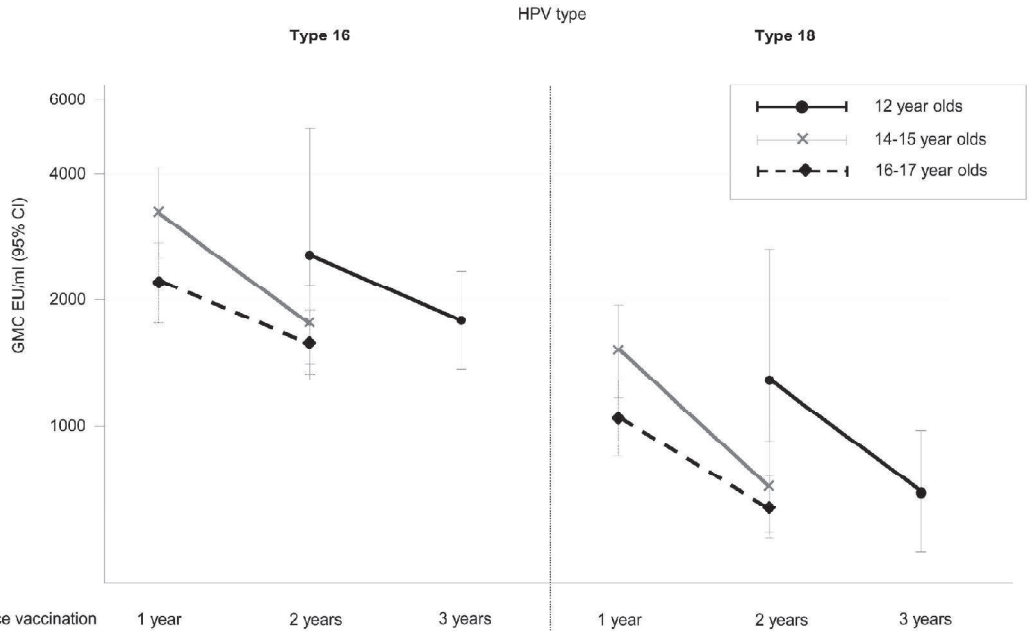


Fig 4. Geometric mean concentrations (GMCs) for HPV type 16 and 18 among those with probable vaccine-induced seropositivity. Stratified by age at HPV vaccination and time since vaccination. Restricted to women with a known date of birth (n = 564). GMC for probable natural infection: 71 EU/mL for type 16; 36 EU/mL for type 18. Time from 1st September in year first offered the HPV vaccine as part of the national immunisation programme to the date serology specimen was taken. Abbreviations: EU/mL = ELISA units per millilitre per millilitre

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unvaccinated females which could have led to some limited misclassification. Our data are consistent with clinical trial data in showing a 10 to 20-fold higher GMC in those with dual seropositivity (presumed largely vaccinated) than those seropositive for only one HPV type (presumed largely natural infections). Those classified as "possible natural infection or vaccine-induced seropositivity" likely reflect the group with natural infection for both HPV types although this proportion is slightly higher than that detected by competitive Luminex assay (cLIA) in a pre-immunisation survey using the same serum collection although a different assay (2.8% in this study compared to 1.8% in 15–19 year old females included in the survey performed prior to the introduction of the HPV immunisation programme [12]).

The average antibody concentrations declined with both increasing age at vaccination and increasing time since vaccination (Fig 4). This is consistent with other observations of immunogenicity by age and the fact that highest levels are reported immediately after HPV vaccination with a slight decrease subsequently [14;15]. Partial vaccination (one or two doses only) was also reportedly more common at older ages of vaccination. We present data up to three years post-vaccination which demonstrate that those with probable vaccine-induced seropositivity still had far greater antibody levels than those following a natural infection. Longer-term serosurveillance, and infection surveillance, is needed to monitor the significance of waning antibody concentrations.

Quantitative antibody concentrations from the study are not comparable to those from studies which use different assays/cut-offs, because currently no international HPV standard reference serum exists, and different laboratories therefore use 'in-house' standard sera making

direct comparisons nonviable. One point for consideration is that the ELISA methodology we applied used an increased lower limit of quantification compared to previous reports (we use a lower limit of 19 EU/mL for HPV16 and 18 EU/mL for HPV18 whereas 8 EU/mL and 7 EU/mL respectively have been used previously) [11]. Using this higher cut-off, 92 women previously considered HPV seropositive were reclassified as seronegative. Although this change resulted in a modest decrease in sensitivity of the ELISA, this had little effect on the classification of specimens as those with natural infection seropositivity or vaccine-induced seropositivity.

Residual serum specimens for this surveillance are taken from females attending for diagnostic and screening tests, hence may not be representative of the general population. The reason for the initial test where the serum sample was taken is not provided to SEU along with the sample. Where it was known that a sample originated from a GUM clinic, this was indicated. Analyses were performed separately for known GUM samples and other samples and results were very similar (data not shown). No data are collected on social deprivation, ethnicity or country of birth of females although since England has free access to health care this reduces the potential bias associated with health-seeking behaviour. One previous paper suggested that the comparable results between coverage data and other vaccine seroconversion rates provide some assurance of the representativeness of these specimens [10].

Vaccination coverage may have been slightly under-reported for the older catch-up vaccination cohorts. In this group, vaccination was largely performed outside of schools and revised estimates of coverage to include data on immunisations given late in 'mop-up' sessions were not readily available in all areas hence the quality of these data was less certain (2). This would be consistent with the greater differences between reported coverage and vaccine-induced seropositivity observed in females offered vaccination at older ages. However, we also consider that the proportion reported to have received only one or two doses of the vaccine is far greater in older ages (just under 5% in those vaccination in the routine cohorts compared to around 15% in the older catch-up cohorts). The majority of females who had received two-doses would have received the first two doses of the vaccine (i.e. with just one or two months between doses). Limited data are available on antibody responses following such a schedule. Data from trials have shown 100% of females seroconvert after two-doses given six months apart with non-inferior antibody concentrations compared to females receiving three-doses [16]. We were therefore unable to distinguish differences in antibody concentrations in females receiving one- or two-doses of the vaccine from females receiving all three doses. Therefore the higher than expected level of vaccine associated seroprevalence in the older cohorts could suggest that three-dose coverage in the catch-up cohorts have been higher than recorded and/or that vaccination of 13–17 year olds with one or two-doses of the vaccine generated high antibody concentrations.

Future studies using these methods will explore serological coverage in certain demographic and behavioural subgroups to identify those relatively lacking in direct vaccine-protection and will monitor antibody levels over longer times since vaccination. This will be an important part of assessing longer term protection.

Supporting Information

S1 Data. Minimal data set. Field names: gender; yearofcollection: Year sample taken; age_year: Age at sample date; result_16: HPV16 antibody concentration (EU/mL); result_18: HPV18 antibody concentration (EU/mL).
(XLSX)

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Author Contributions

Conceived and designed the experiments: ES RB. Performed the experiments: ES JF RW RB. Analyzed the data: DM KS. Wrote the paper: DM KS. Coordinated collection of residual sera: RP ES. Provided details of reported HPV vaccination coverage: JW SD. Provided input and advice on SEU serology testing: RP.

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RESEARCH PAPER COVER SHEET

PLEASE NOTE THAT A COVER SHEET MUST BE COMPLETED FOR EACH RESEARCH PAPER INCLUDED IN A THESIS.

SECTION A – Student Details

Student	David Mesher
Principal Supervisor	Sara Thomas
Thesis Title	Assessment of the population-level impact of a high coverage HPV immunisation programme in young females

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

Where was the work published?	Public Health England: Health Protection Report		
When was the work published?	October 2016		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	N/A		
Have you retained the copyright for the work?*	**Yes**	Was the work subject to academic peer review?	**No**

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SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	
Please list the paper's authors in the intended authorship order:	
Stage of publication	Choose an item.

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	The Public Health England (PHE) Seroepidemiology Unit (SEU) is a source of residual sera archived for surveillance activities and held at the PHE Vaccine Evaluation Unit, established before the start of my PhD. The initial idea to use residual sera to monitor HPV vaccination uptake was developed by Kate Soldan, Ray Borrow and

	Richard Pebody. Laboratory testing was performed by Elaine Stanford, Jamie Findlow, Rosalind Warrington and Ray Borrow. I designed and conducted the data analysis, including developing techniques to ascertain HPV vaccination status from results of HPV serology testing. I wrote the first draft of this paper which was commented on by all authors.

Student Signature: [redacted] **Date:** 12/04/2018

Supervisor Signature: [redacted] **Date:** 13/04/18

Public Health England

Health Protection Report
weekly report

Infection report

Volume 10 Number 34 Published on: **7 October 2016**

HIV-STIs

Post-immunisation monitoring of HPV vaccine-induced seroprevalence in England 2010 to 2013

Background

Seroconversion occurs following an estimated 50-70% of incident natural human papillomavirus (HPV) infections in women [1-4] and natural infection often elicits only a weak antibody response. Conversely, vaccination induces seroconversion in ~100% of HPV-naïve recipients and generally results in far higher antibody concentrations than those following natural infection [5,6]. As such, serological assays which provide a quantitative measure of the level of HPV type-specific antibodies can be used to estimate HPV vaccination coverage.

Public Health England (PHE)'s monitoring of HPV seroprevalence (as part of work to monitor and evaluate the National HPV Immunisation Programme) has begun with a study of young women in the first birth cohorts to be offered HPV immunisation, primarily to compare vaccine-induced seroprevalence to nationally reported coverage data. The first results from this surveillance have been published previously with data from 2,146 specimens collected between 2010 and 2011 [7]. We report here updated findings with results from 3,772 specimens collected up to 2013.

Methods

Residual serum specimens were collected for 15-19 year old females from the PHE Seroepidemiology Unit (SEU). SEU specimens are collected from individuals attending for microbiological and/or biochemical tests. Serum samples were submitted with data on gender, age at collection and year of collection from fourteen laboratories in England. Laboratories were asked to identify, if possible, any specimens collected via Genitourinary (GU) Medicine clinics (defined as No, Yes or Not known). Specimens collected from 2010 to 2013 are included in this analysis.

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Where date of birth was available, this was used to generate the age and calendar year that HPV vaccination would have been offered: this was available for 2355/3772 (62.4%) of women. For the remainder, with age in years available, likely year of eligibility for HPV vaccination was estimated. Specimens collected in January-March following the due date of first vaccine dose were excluded in order to study seroprevalence after, not during, the scheduled full course of vaccination.

Specimens were tested for antibodies to HPV types 16 and 18 using a type-specific ELISA. Testing was performed at the PHE Vaccine Evaluation Unit (VEU), Manchester. Specimens were considered to be seropositive above cut-offs determined previously with this assay: 19 and 18 ELISA units per millilitre (EU/mL) for HPV 16 and 18, respectively.

Methods to determine vaccine-induced seropositivity were as previously described [7]. In brief, each result was classified as “low”, “moderate” or “high” based on the concentration of HPV antibodies for HPV16 and HPV18. Specimens were then categorised as (i) “probable” vaccine-induced seropositivity if seropositive for both types with high concentration for at least one type or moderate concentrations for both types, (ii) “probable” natural infection if seropositive for one type only, (iii) “possible” natural infection or vaccine induced seropositivity if low seropositivity for both types or low seropositivity for one type and moderate for the other. Antibody concentrations are presented as geometric mean concentrations (GMCs) among seropositive specimens.

Routinely published data on HPV vaccine coverage in England has been reported by academic year. To compare these data with seroprevalence we estimated coverage by year of age and calendar year.

Results

A total of 4,045 specimens had a valid result for type-specific HPV antibodies for both HPV types 16 and 18. Excluding 323 samples which were collected in the January to March of the year following the due date of first vaccine dose; 3,722 specimens were included in this analysis (1205, 941, 952 and 674 collected in 2010, 2011, 2012 and 2013, respectively). The mean age of women providing a specimen was 17.8 years (SD 1.42 years). Overall, just under one-third (32.4%) of all specimens were identified as coming from a GU setting although this was not known for the majority of other specimens (64.1%): specimens from a known non-GU setting had higher seroprevalence (p=0.01 for vaccine-induced seropositivity). Table 1 shows the demographics of all eligible women alongside the proportion seropositive for at least one HPV type.

Table 1. Seropositivity by clinical setting, age and laboratory sending specimen

	Number with valid result	Proportion seropositive for HPV 16 and/or 18	Proportion seropositive for HPV 16 and 18	Vaccine-induced seropositivity
	n	n (%)	n (%)	n (%)
Total	*3,772*	*2,861 (75.8)*	*2,638 (69.9)*	*2,472 (65.5)*
Genito-urinary Medicine (GUM) clinic setting				
Yes	1,206	928 (76.9)	852 (70.6)	798 (66.2)
No	149	126 (84.6)	119 (79.9)	114 (76.5)
Unknown	2,417	1,807 (74.8)	1,667 (69.0)	1,560 (64.5)
Age specimen taken				
15 years	643	527 (82.0)	512 (79.6)	492 (76.5)
16 years	729	600 (82.3)	573 (78.6)	547 (75.0)
17 years	590	464 (78.6)	433 (73.4)	401 (68.0)
18 years	957	713 (74.5)	645 (67.4)	600 (62.7)
19 years	853	557 (65.3)	475 (55.7)	432 (50.6)
Laboratory[1]				
North East				
Newcastle	361	305 (84.5)	280 (77.6)	258 (71.5)
North West				
Manchester	577	443 (76.8)	413 (71.6)	395 (68.5)
Yorkshire and The Humber				
Leeds	946	729 (77.1)	671 (70.9)	624 (66.0)
East Midlands				
Cambridge	166	116 (69.9)	107 (64.5)	100 (60.2)
Leicester	347	285 (82.1)	277 (79.8)	264 (76.1)
West Midlands				
Birmingham	81	51 (63.0)	43 (53.1)	41 (50.6)
London				
Barts and The London	230	134 (58.3)	121 (52.6)	108 (47.0)
St George's Hospital	167	99 (59.3)	83 (49.7)	77 (46.1)
PHL London[2]	139	96 (69.1)	82 (59.0)	76 (54.7)
South Central				
Southampton	103	85 (82.5)	79 (76.7)	74 (71.8)
South East				
Brighton[3]	49	30 (61.2)	25 (51.0)	24 (49.0)
South West				
Bristol	69	58 (84.1)	55 (79.7)	51 (73.9)
Exeter	510	412 (80.8)	384 (75.3)	363 (71.2)
Gloucester	27	18 (66.7)	18 (66.7)	17 (63.0)

1: Proportion seropositive for each laboratory are age and year-standardised

2: PHL = Public Health Laboratory; specimens collected in 2012 and 2013 only

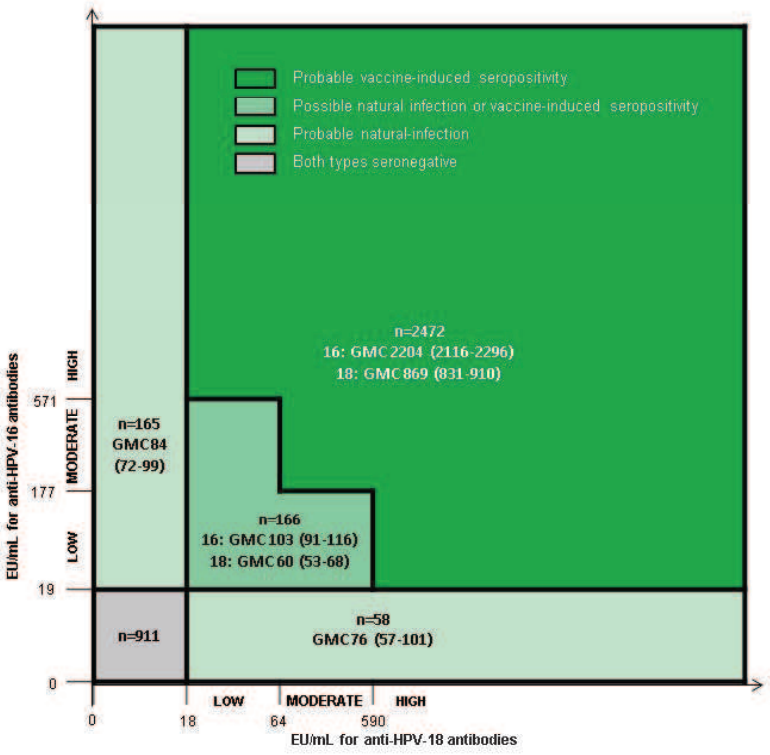
3: Specimens collection in 2010 only

A total of 69.9% (2,638/3,772) of specimens were seropositive for both types HPV16 and HPV18. Seropositivity for HPV16 only and for HPV18 only was found in 4.4% (165) and 1.5% (58) of specimens respectively (table 2; figure 1). Antibody concentrations were generally far higher for specimens seropositive for both HPV types than amongst those seropositive for only one type (median 2017.5 EU/ml vs 70 EU/ml for HPV16 and 804.5 EU/ml vs 59 EU/ml for HPV18) (table 2).

Table 2. Antibody concentrations for types HPV16 and HPV18

		HPV type 16		HPV type 18	
HPV type	n (%)	Median EU/mL (IQR)	95% range	Median EU/mL (IQR)	95% Range
Both types negative	911 (24.2%)	–	–	–	–
16 negative, 18 positive	58 (1.5%)	–	–	59 (30-172)	18 – 590
16 positive, 18 negative	165 (4.4%)	70 (37-156)	23-571	–	–
Both types positive	2,638 (69.9%)	2,017.5 (929-4200)	177 – 11,675	804.5 (343-1,756)	64 – 5,460
Total	3,772 (100%)				

Figure 1. Classification of vaccine-induced seropositivity (n=3,772)



Using the range of concentrations for types 16 and 18 seropositives we classified each result as, (i) "high" seropositivity if the result was above the 95% range of concentrations among those with a single antibody (i.e. unusually high for presumed largely naturally infected); (ii) "low" seropositivity as below the lower 95% range of concentrations among those seropositive for both HPV types (i.e. unusually low for dual seropositivity, presumed largely immunised); (iii) "moderate" seropositivity as between these two values.

Vaccine-induced seropositivity was highest in the younger ages with higher expected vaccine coverage (table 3). This finding was consistent in sub-analyses by region (data not shown). The overall proportion of females with probable vaccine-induced seropositivity was 66% (2,472/3,772) and 4.4% (166/3,772) with possible natural infection or possible vaccine-induced seropositivity. The proportion of females with vaccine-induced seropositivity was slightly lower than the reported three-dose coverage for 15 and 16 year olds but higher at older ages (table 3 and figure 2).

Table 3. Seropositivity for HPV 16 and 18 amongst all specimens tested for both HPV types, by age

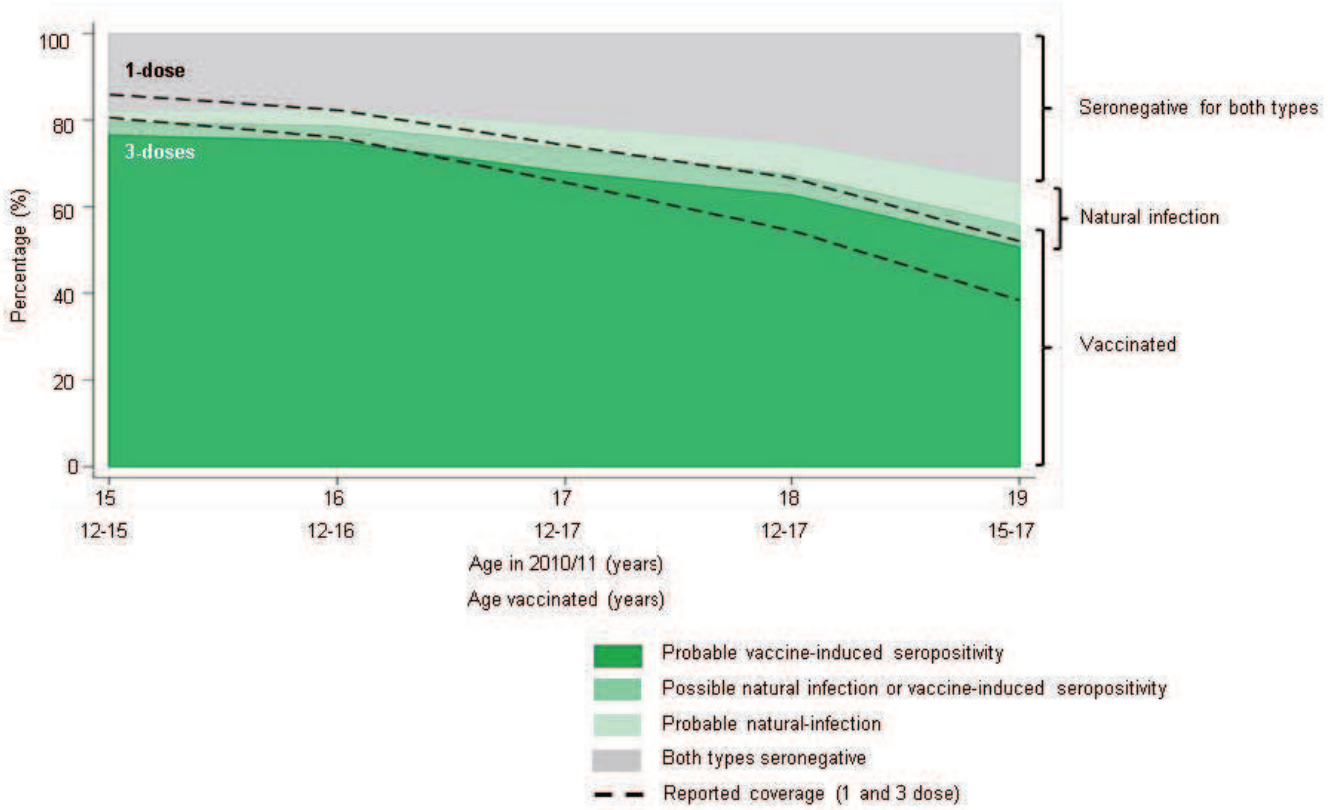
	Age in years					
HPV type	15	16	17	18	19	*Total*
Both types negative	18.0% (116)	17.7% (129)	21.4% (126)	25.5% (244)	34.7% (296)	*24.2% (911)*
Natural infection seropositivity:						
- Probable 18 only	1.1% (7)	1.2% (9)	1.0% (6)	2.0% (19)	2.0% (17)	*1.5% (58)*
- Probable 16 only	1.2% (8)	2.5% (18)	4.2% (25)	5.1% (49)	7.6% (65)	*4.4% (165)*
- Probable 18 or 16	2.3% (15)	3.7% (27)	5.3% (31)	7.1% (68)	9.6% (82)	*5.9% (223)*
- Probable and possible	5.4% (35)	7.3% (53)	10.7% (63)	11.8% (113)	14.7% (125)	*10.3% (389)*
Vaccine-induced seropositivity:						
- Probable	76.5% (492)	75.0% (547)	68.0% (401)	62.7% (600)	50.6% (432)	*65.5% (2,472)*
- Probable and possible	79.6% (512)	78.6% (573)	73.4% (433)	67.4% (645)	55.7% (475)	*69.9% (2,638)*
Expected 1-dose (national)	86.0%	82.3%	74.2%	66.7%	52.1%	-
Expected-3-dose (national)	80.6%	76.0%	65.6%	54.5%	38.4%	-

The GMCs amongst all women and those with probable vaccine-induced seropositivity group declined slightly with age (table 4). Furthermore, in women with a known date of birth, the GMCs declined after the first year but then seemed to remain stable at a level far higher, on average, than the GMCs for those seropositive for only one HPV type (figure 3).

Table 4: Geometric mean concentrations (GMCs; 95% CI) of EU/mL for HPV16 and HPV18, by age

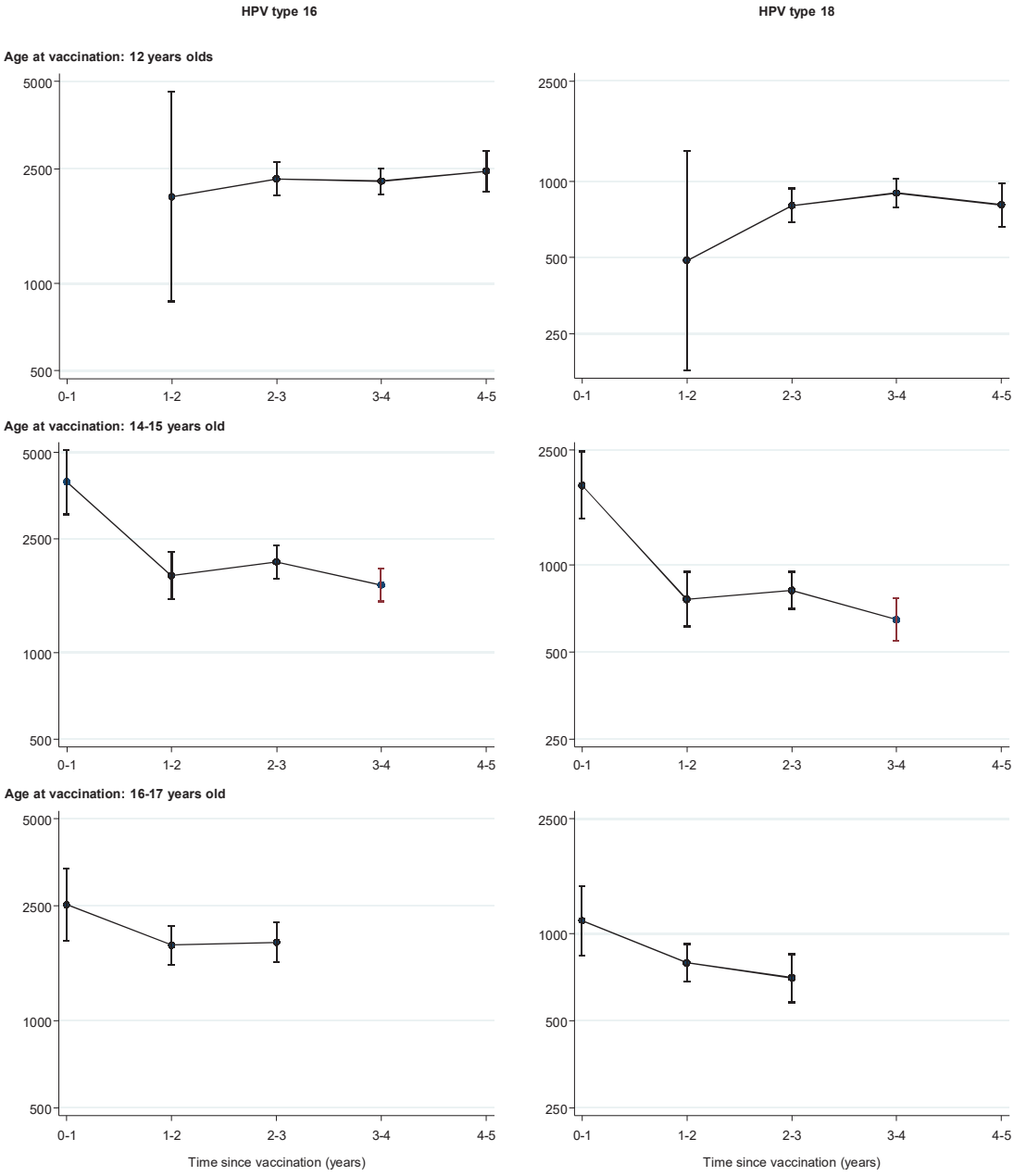
	Age in years				
	15	16	17	18	19
Type 16					
Seropositive	2,207 (1,979-2,461)	1,840 (1,661-2,037)	1,424 (1,246-1,628)	1,357 (1,213-1,517)	1,042 (911-1,193)
Vaccine-induced seropositive	2,632 (2,402-2,883)	2315 (2,134-2,513)	2,078 (1,871-2,309)	2,087 (1,920-2,269)	1,929 (1,750-2,125)
Type 18					
Seropositive	857 (766-959)	792 (714-878)	664 (585-753)	631 (569-699)	589 (520-668)
Vaccine-induced seropositive	993 (896-1,100)	925 (841-1,017)	819 (730-919)	814 (744-891)	799 (715-894)

Figure 2. Published HPV vaccine coverage and vaccine-induced seropositivity, by age (n=3,772)



"Probable" vaccine-induced seropositivity defined as seropositive for both types with high concentration for at least one type or moderate concentrations for both types. "Probable" natural infection as seropositive for one type only. "Possible" natural infection or vaccine-induced seropositivity defined as low seropositivity for both types or low seropositivity for one type and moderate for the other

Figure 3. Geometric mean concentrations (GMCs; EU/mL) and 95% CI for HPV16 and HPV18 among those with probable vaccine-induced seropositivity (restricted to women with a known date of birth who would have been eligible for vaccination as part of the national immunisation programme (n=1,569)



Discussion

We have previously reported that serological surveillance confirms high vaccine coverage of the HPV vaccination programme in young females in England, particularly in those offered the vaccine at younger ages, but that there was a slightly higher proportion with vaccine-induced seropositivity compared to reported three-dose coverage in females offered HPV vaccination at an older age suggesting that three-dose coverage in the catch-up cohorts could be higher than reported and/or that two-dose coverage at these ages is associated with high antibody responses. These updated analyses strengthen these conclusions. In addition, we demonstrate that whilst geometric mean antibody concentrations declined immediately after vaccination, levels then remained fairly stable up to five years post-vaccination. Furthermore, the average antibody concentrations were still far greater than antibody concentrations following a natural HPV infection.

Vaccine status of women in this study is unknown which leads to two important limitations. Firstly, vaccine-induced seropositivity can't be compared to recorded vaccination status, hence we must assume that these women are representative of the general population with similar HPV vaccination coverage to national reported data. Residual serum specimens for this surveillance are taken from females attending for diagnostic and screening tests. No additional demographic data are collected on social deprivation, education, ethnicity or other factors which may be associated with vaccine uptake. However, everyone in England has free access to health care which reduces the potential bias associated with health-seeking behaviour and previous studies have suggested that results are comparable for other vaccines [8]. Secondly, measuring changes in natural infection among unvaccinated women compared to similar surveys conducted prior to the introduction of vaccination would allow us to consider if there is evidence of a herd protection effect. However, a limitation of this analysis was that it wasn't possible to accurately distinguish between women with a natural infection and women who have been vaccinated.

Among those vaccinated between 14 to 17 years of age, these results show initial waning of antibody concentrations immediately following vaccination and then stabilisation, which is fairly consistent with results from clinical trials [5]. It wasn't possible to consider waning after vaccination in the routinely vaccinated cohorts as sera from 12-14 years olds were not included in this analysis. Whilst the level of protection required to prevent HPV infection and related disease is not known, the plateau of antibody concentrations is still far higher than those seen with a natural infection for all ages. As such, annual monitoring may not be essential but periodic surveillance to monitor that antibody concentrations are remaining high in the general population could be valuable, and to check antibody concentrations in

recipients of the two dose schedule in due course. Future studies should also consider variations in the proportion of women with vaccine-induced seropositivity from different subgroups which will be required to accurately monitor of the impact of HPV vaccination.

Conclusion

These data add to previous data confirming high coverage of HPV vaccination in England but with some potential under-reporting of vaccination of older females and/or a potential protective effect of receiving fewer than three doses. This updated analysis provides data on antibody responses up to five years post-vaccination. Whilst there is some evidence of slight declines in antibody concentrations over time since vaccination, these still remain far higher than antibody concentrations following a natural infection.

Authors

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RESEARCH PAPER COVER SHEET

PLEASE NOTE THAT A COVER SHEET MUST BE COMPLETED FOR EACH RESEARCH PAPER INCLUDED IN A THESIS.

SECTION A – Student Details

Student	David Mesher
Principal Supervisor	Sara Thomas
Thesis Title	Assessment of the population-level impact of a high coverage HPV immunisation programme in young females

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

Where was the work published?			
When was the work published?			
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
Have you retained the copyright for the work?*	Choose an item.	Was the work subject to academic peer review?	Choose an item.

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SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	This is not yet decided
Please list the paper's authors in the intended authorship order:	David Mesher, Sara L Thomas, Ezra Linley, Claire Edmundson, Marta Checchi, Tim Waterboer, Noemi Bender, Simon Beddows, Ray Borrow, Kate Soldan
Stage of publication	**Not yet submitted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I developed, with Kate Soldan, the initial proposal for this surveillance and for the case-control study. I wrote the protocol and designed the study, including processes to collect specimens and data. Kate Soldan and Sara Thomas commented on the proposed

	sampling technique for specimen collection. I recruited local laboratories for participation and worked with them to ensure all relevant approvals and agreements were in place. I was responsible for managing the data collection. I established processes to link data with specimen results and anonymise records prior to testing. I was responsible for conducting this linkage/anonymisation for the first years of the surveillance and then oversaw this process for latter years. I liaised with the laboratory performing the HPV testing to inform them when specimens could be tested and to approve techniques for specimen receipt. Specimen receipt and initial processing was established and conducted by Ezra Linley and Ray Borrow. Specimen testing was performed by Tim Waterboer and Noemi Bender. I designed and conducted all data management and analysis for the study. Kate Soldan and Sara Thomas gave advice on the statistical analysis. I wrote the first draft of the manuscript which was commented on by other authors.

Student Signature: [redacted] **Date:** 12/04/2018

Supervisor Signature: [redacted] **Date:** 13/04/18

Title: Post-vaccination HPV seroprevalence among female sexual health clinic attenders in England

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Abstract

Background: The National HPV Immunisation Programme was introduced in England in September 2008 using the bivalent vaccine. We used serological surveillance to consider variations in HPV vaccination uptake by patient characteristics. We also conducted a case-control study to consider the effect of the bivalent vaccine against genital warts.

Methods: We collected residual serum specimens from 16-20 year old women attending a sexual health clinic in England for an HIV and/or syphilis test. Sera were tested for antibodies against HPV16 and HPV18 using a GST L1-based multiplex serology assay. Patients were classified as having vaccine-induced seropositivity if they were seropositive for both HPV16 and HPV18.

We compared differences in vaccine-induced seropositivity by patient characteristics using a weighted logistic regression model. For the case-control study, cases and controls were selected using two approaches; exclusive sampling and concurrent sampling. We conducted an unconditional logistic regression adjusted for matching variables for all women and a separate matched analysis which included only cases and controls selected using concurrent sampling.

Results: A total of 3,959 (99.6%) serum specimens had a valid result for both HPV types. The proportion of women with vaccine-induced seropositivity decreased with age (from 72.4% in 16 year olds to 44.8% in 20 year olds). We also demonstrated lower vaccine-induced seropositivity among women born outside the UK, women from more deprived areas and women with a history of chlamydia diagnosis. A difference in uptake by ethnic group was also seen but this was largely explained by differences in deprivation and country of birth. There was no evidence of a protective effect of the HPV vaccine against genital warts (adjusted odds ratio of 1.05; 95% CI 0.84 to 1.29 for all women. adjusted odds ratio of 1.02; 95% CI 0.72 to 1.45 restricted to cases and controls selected using concurrent sampling).

Discussion: Our results do not support a cross-protective effect of the bivalent vaccine against genital warts. Vaccine-induced seropositivity in this high-risk population did demonstrate lower HPV vaccination uptake in some sub-groups.

Introduction

The National HPV Immunisation Programme was introduced in the UK in September 2008. Initially the bivalent vaccine was used with routine vaccination of 12-13 year olds and a catch-up programme in the first two years of vaccination for all females up to the age of 18 years. All vaccinations as part of the national programme were offered free of charge. Vaccination of routine cohorts and younger catch-up cohorts was almost exclusively offered in schools whereas vaccination of older catch-up cohorts was offered in different primary care and education settings and varied by local area.

Monitoring of national HPV vaccination coverage in England relies on aggregated numerator and denominator data reported to Public Health England (PHE) from local areas. These national data have shown high vaccine coverage of over 80% for all routine cohorts[78]. Coverage among the catch-up cohorts was more variable depending on the age the vaccine was offered (3-dose coverage ranging between 39% and 76%)[78]. There are two main limitations of this method of monitoring vaccine coverage. Firstly, these data rely on accurate recording of vaccine doses given at local areas which, particularly in the older catch-up cohorts, are often derived by collating data from different data systems and different settings. Secondly, the national data are stratified by academic year (i.e. birth cohort) but not by ethnicity, sexual risk or any other factors. We have previously investigated the first of these two limitations by developing a robust technique to monitor HPV vaccine-induced seropositivity using serological testing of residual specimens from a broadly population based survey conducted in England. These results confirmed the high vaccine coverage among young women although there was some evidence of slightly higher vaccine-induced seropositivity in the older cohorts compared to the nationally published vaccine coverage[166]. In this paper, we aim to address the second limitation of the national published HPV vaccine coverage by considering

the equity of HPV vaccination uptake among women at higher risk of STIs including HPV. This is important to ascertain whether there has been lower vaccination uptake in those who may be at higher risk for HPV (and therefore cervical cancer) and to allow more accurate predictions of the impact of HPV vaccination on disease incidence in the future. We have investigated HPV seroepidemiology in young women attending sexual health clinics in England (i.e. at higher risk of STIs) and stratified vaccine-induced seropositivity by age, ethnicity, country of birth, index of multiple deprivation (IMD) and current or previous history of other STIs.

Additionally, we have conducted a nested case-control study to investigate the effect of vaccination with the bivalent vaccine on the incidence of genital warts. Whilst a cross-protective effect of the bivalent vaccine against low-risk HPV types which cause genital warts was not initially expected, ecological observations in England have shown moderate declines in diagnoses of genital warts since the introduction of the bivalent vaccine that were associated with vaccination coverage by age[84, 159]. Also, a post-hoc analysis of the PATRICIA trial reported efficacy against HPV6/11[74]. Together, these findings raised a hypothesis that the bivalent vaccine confers some moderate cross-protective effect against genital warts. To test this hypothesis, and so inform vaccine choice in the future, we designed a nested case-control study within our serosurveillance. This was done by sampling cases and controls before the quadrivalent vaccine was introduced into the National HPV Immunisation Programme in 2012.

Methods

Eligible population and specimen collection

The GUMCAD STI Surveillance System is held and managed at PHE and collects information on all attendances, STI tests and diagnoses at sexual health services in England. We made use of this data collection system to identify eligible attendances

for the purposes of our HPV surveillance. Specifically, eligible women were aged 16-20 years old and had attended for an HIV and/or syphilis test at one of six sexual health clinics across England between 2011 and 2015; Cheltenham General Hospital, Gloucester Royal Hospital, Nottingham City Hospital, Royal Hallamshire Hospital, Homerton Hospital and Countess of Chester. These women would have been eligible for their first HPV vaccination dose between 2008 and 2012, when aged between 12 and 18 years old (Table 1): the bivalent vaccine was offered through the National HPV Immunisation Programme for all of this time period.

We randomly selected eligible attendances to meet a pre-defined target number of specimens (see sample size below) for each age-group and clinic. HIV and syphilis testing for these women was performed at five local laboratories (testing for Cheltenham General Hospital and Gloucester Royal Hospital was performed at the same local laboratory) and residual specimens were frozen and held for at least two years following this test at each laboratory. Therefore, we were able to retrospectively request residual sera specimens directly from each testing laboratory. We sent local laboratories a list of patient IDs and the attendance dates of the eligible HIV or syphilis test. Laboratories were asked to identify the residual serum specimen associated with these attendances. If no specimen was taken on the exact attendance date but there was a specimen within 7 days (either direction) then the laboratories were asked to provide this specimen.

The five laboratories were requested to provide rigid polypropylene serum vials with a screw-cap with O-ring seal, with a capacity of no more than 2mL. A minimum volume of 250µl was requested for each specimen. Serum samples were labelled only with the patient ID and attendance date. Residual specimens were sent to the PHE Vaccine Evaluation Unit (VEU) for processing. On receipt at VRD, samples were relabelled with a unique HPV study number which was electronically linked with the patient ID and attendance date.

Data collection

Prior to HPV testing, additional GUMCAD data were linked to the HPV study number; ethnicity (categorised as white, black, Asian, mixed and other (including Chinese and any other ethnicity)), country of birth, index of multiple deprivation (based on lower layer super output area (LSOA) of patient where available (93% of patients) or otherwise LSOA of clinic (7% of patients)), and whether a patient had a concurrent or previous diagnoses of gonorrhoea, herpes or chlamydia.

Following linkage, samples were pseudonymised prior to release for HPV testing by discarding patient ID and attendance date, keeping only the unique HPV study number.

Case-control study

Prior to requesting specimens, we determined the case and control status using data from GUMCAD. Cases were defined as women with a diagnosis of a first episode of genital warts (i.e. assumed to be an incident case). Controls were defined as women with no current or previous diagnoses of genital warts since 2008 (i.e. the date that GUMCAD data were first collected).

Cases and controls selected from specimens taken between January 2011 and June 2012 were selected using exclusive sampling, meaning that once selected, cases and controls could not be reselected. Three controls from the same laboratory and age were selected for each case. Cases and controls selected from specimens taken between July 2012 and December 2015 were selected slightly differently. Three controls were matched to each case (on testing laboratory, quarter and year of sample collection and age) using a concurrent sampling method. Specifically, controls were selected from all women who had no warts diagnosis (or previous diagnoses) in the same quarter/year that the case was diagnosed. Cases could have been selected as controls prior to their first warts diagnosis. Similarly,

controls could be selected more than once on different attendances. This change in sampling was to allow estimation of a rate ratio (rather than odds ratio).

Patient consent

This surveillance made use of residual specimens taken originally for other purposes. Specimens were unlinked from any patient identifiable information prior to being tested for the purposes of Public Health Monitoring. As such, patient consent was not required.

HPV serology testing and determining vaccine-induced seropositivity

The 2mL Eppendorf specimen tubes were sent in dry ice to DKFZ, labelled only with the unique HPV study number and no other patient identifiers. Multiplex serology was performed as described previously[150]. Briefly, multiplex serology is a fluorescent bead-based assay allowing for analysis of antibody responses to several antigens in one reaction. Antigens were expressed as Glutathione S-transferase (GST) fusion proteins and affinity-purified on glutathione-derivatized polystyrene beads (Luminex Corp, Austin, TX, USA). Different antigens were purified on different bead sets as defined by the beads' internal fluorescence. The antigen-loaded bead sets were mixed and incubated with serum. A Luminex flow cytometer then distinguished between the bead sets, and therefore the loaded antigen, as well as quantified the amount of bound serum antibody by a human IgG secondary antibody and Streptavidin-R-phycoerythrin fluorescent reporter conjugate. The output was the median reporter fluorescence intensity (MFI) of at least 100 beads per set per sample. Net MFI were generated by subtracting two background values resulting from a blank (a well containing no serum but antigen-loaded beads and all secondary reagents) as well as from a bead set loaded with GST only. Antigen-specific cut-offs were defined by visual inspection of frequency distribution curves (percentile plots) at the approximate inflection point of the curve to dichotomize

antibody responses as seropositive and seronegative as previously described[151-155]. A cut-off of 100 MFI was used to determine seropositivity for the L1 proteins of both HPV16 and HPV18. As a sensitivity analysis, we considered different cut-offs for seropositivity of 80 MFI and 120 MFI.

Patients were classified as having vaccine-induced seropositivity if they were seropositive to L1 proteins for both HPV16 and HPV18.

Data analysis

The proportion of women who had vaccine-induced seropositivity was weighted to account for the over-sampling of women who had a history of genital warts for the nested case-control study. Weights were calculated for each year and age according to the probability of selection from the total number of attendances with and without a diagnosis of genital warts (using the full GUMCAD dataset for the clinics and years included in this surveillance). The proportion with vaccine-induced seropositivity was presented alongside the expected HPV vaccination coverage. Expected coverage was estimated for this population by applying national age and year-specific 3-dose HPV vaccination coverage estimates from published data[78] and calculating an average by dividing by the total number of eligible women included in this surveillance. The expected uptake of the first HPV vaccine dose, based on national published data, was also estimated for this survey population in a similar way.

Weighted logistic regression was used to explore the differences in vaccine-induced seropositivity by age, ethnicity, country of birth, IMD quintile and presence of an STI. All variables were included in the multivariable logistic regression analysis. Year of specimen collection was also included in the multivariable regression model to adjust for potential confounding by calendar period. Results were presented as unadjusted and adjusted odds ratios (ORs) and confidence intervals (95% CI).

Exact date of birth and HPV vaccination cohort was unknown for women included in this surveillance and hence we were unable to determine the exact year and age that each woman would have been offered the HPV vaccine as part of the National HPV Immunisation Programme. However, based on the age and year of sample collection, it was possible to ascertain a range of ages which a woman could have been offered the HPV vaccine (Table 1). The above analyses were stratified for women for whom it could be determined (with certainty) that they fell within either the routine cohorts or the catch-up cohorts.

For the case-control study, we conducted two analyses. The first analysis included all cases and controls (i.e. those selected by exclusive sampling and concurrent sampling). We performed an unconditional logistic regression adjusted for matching variables: quarter and year of specimen collection, age and laboratory[165]. To control for potential confounding between case/control status and vaccination status, we also adjusted for ethnic group, country of birth, quintile of IMD and presence of an STI. Unadjusted and adjusted odds ratios and 95% confidence intervals were presented. The second, matched, analysis included only cases and controls selected by concurrent sampling (from July 2012 onwards). Controls were excluded from this analysis if the specimen from their matched case was not available, and similarly cases were excluded if none of the specimens from their three matched controls were returned. If at least one matched control was returned for a case, then these cases and controls were included in the analysis (see Figure 1). We performed a conditional logistic regression for a matched analysis. As above, the conditional multivariable regression model was adjusted for ethnic group, country of birth, quintile of IMD and presence of an STI. Unadjusted and adjusted odds ratios (which, given the concurrent sampling, estimated rate ratios) and 95% confidence intervals were presented.

For both sets of analyses, individuals with missing data for variables included in the multivariable model were excluded from the analysis (i.e. a complete-case analysis).

Sample size

We powered our surveillance to compare HPV vaccine-induced seropositivity between population subgroups (ethnicity [categorised as white, black and Asian/mixed/other combined]; quintile of deprivation and whether a patient had a current or previous STI). The largest required sample size was to compare coverage in white women (~77% of attenders) with Asian/mixed/other women (~9% of attenders). To detect a 7.5% absolute difference in vaccine-induced seropositivity among Asian/mixed/other women (compared to an estimated 55% coverage among the reference group, white women), with alpha=0.05 and beta=0.2, required a sample size of 4,311 women (~3,319 white women, ~604 black women, ~388 Asian/mixed/other women).

The nested case-control study was powered to identify a vaccine effectiveness of the bivalent vaccine against genital warts of 30%. This gave a required sample size of 333 cases (assuming approximately 50% of controls were vaccinated, with alpha=0.05 and beta=0.2). We increased the number of samples to allow for multivariable regression analysis (15%) which gave a final sample size of 383 cases (and 1,149 matched controls). We requested 420 cases (and 1,260 controls by concurrent sampling) to allow for approximately 10% of residual samples that couldn’t be retrieved or had an inadequate serology result.

Results

Data and sample collection

A total of 3,973/4,888 (81.3%) requested serum specimens were retrieved and returned for serology testing (Figure 1). Of these, 3,959 (99.6%) had a valid result

for both HPV 16 L1 antibodies and HPV 18 L1 antibodies and were included in the analysis. There were 191 women with missing data (for either ethnicity (n=64), quintile of IMD (n=1) or country of birth (n=149)) and hence these women were excluded from regression analyses.

Estimation of HPV vaccine-induced seropositivity

The age at which HPV vaccination would have been offered by age and year of sample collection is shown in Table 1. The overall proportion of women with vaccine-induced seropositivity was 65.2% compared to expected 1-dose and 3-dose vaccination coverage of 74.3% and 65.8% respectively (based on national published data). As expected, the proportion of women with vaccine-induced seropositivity was higher in younger women who would have been vaccinated at younger ages (72.4% in 16 year olds, 73.1% in 17 year olds, 68.3% in 18 year olds, 61.3% in 19 year olds and 44.8% in 20 year olds; Table 2). This surveillance was originally powered to consider differences in vaccine-induced seropositivity between women of white ethnicity vs. the combined group of Asian women, women of mixed ethnicity and women of other ethnicity. However, we found that the vaccine-induced seropositivity varied widely between Asian women and women of mixed and other ethnicity (with lower vaccine-induced seropositivity in women of mixed ethnicity and of other ethnicity but similar in Asian women compared to white women, Table 2); therefore, we did not consider it appropriate to combine their results. Vaccine-induced seropositivity was also lower in black women compared to white women. However, after adjustment for quintile of deprivation and country of birth, these differences in vaccine-induced seropositivity by ethnic group were diminished (Figure 2 and Supplementary Table 1). In contrast, the lower vaccine-induced seropositivity among women born outside of the UK persisted (adjusted OR; 0.58 (0.44-0.75)), and clear evidence remained of lower vaccine-induced seropositivity in more deprived quintiles (p-value for trend<0.0001). Vaccine-induced seropositivity

was also lower in women who had a current or previous diagnosis of gonorrhoea or chlamydia, but again this was attenuated after adjustment for other factors (Figure 2).

The analysis of this surveillance was not powered to consider differences in vaccine-induced seropositivity by patient characteristics when stratified by age at vaccination. For completeness, these data are included in Figure 2. For women offered the vaccine as part of the catch-up (at 14-18 years old), similar to all women, there was noticeable lower vaccine-induced seropositivity in older ages, women born outside of the UK and in women from more deprived areas. This was less clear for women offered the vaccine routinely (at 12-13 years old) although numbers were small.

Case-control study to consider the effect of the bivalent vaccine against genital warts

Analysis of all returned cases and controls (553 cases and 1,548 controls) gave an unadjusted odds ratio for vaccination against genital warts diagnoses of 1.08 (95% CI 0.88 to 1.34) (Table 3). After adjustment for potential confounding, there remained little evidence for a protective effect of the vaccine against genital warts (adjusted OR 1.05; 95% CI 0.84 to 1.29). The odds ratios from the matched analysis of cases and controls selected using concurrent sampling (thus estimating rate ratios; including 281 cases and 644 controls) similarly, showed little evidence of any association between vaccine-induced seropositivity and the rate of genital warts diagnoses (unadjusted OR 1.05; 95% CI 0.75 to 1.46, and adjusted OR 1.02; 95% CI 0.72 to 1.45).

Sensitivity analyses

Sensitivity analyses using different cut-offs for seropositivity for HPV16 and HPV18 (80 MFI and 120 MFI) changed the overall proportion of vaccine-induced

seropositivity only slightly (from 65.2% to 68.4% and 62.4% respectively). The results of variations by patient characteristics and the odds ratio for vaccination against genital warts were similar to the main analysis using these cut-offs (results not shown).

Discussion

We present two analyses in this paper. Firstly, we have considered HPV vaccine-induced seropositivity among young women attending sexual health services and variations in uptake by certain characteristics. Secondly, with a nested case-control study, we have explored the hypothesis, born from earlier ecological observations and a post-hoc analysis of clinical trial data, that vaccination with the bivalent vaccine confers a protective effect against genital warts.

The overall vaccine-induced seropositivity in this survey was 65.2% but, as expected, this varied by year of collection and age of the woman. Applying age and year-specific vaccination coverage estimates from published data[78] the results for the women included in our surveillance gave an expected 3-dose vaccination coverage of 65.8%. This suggests that vaccine-induced seropositivity in higher-risk women attending for HIV and syphilis testing at sexual health clinics is similar to the 3-dose coverage in the general population. However, the expected proportion of women who would have received one or more doses according to national published data was 74.3%. Unfortunately, in our surveillance we were not able to distinguish between women who were partially vaccinated vs. fully vaccinated. Considering the likelihood that partial vaccination would elicit an immune response in some women (i.e. some of the women with vaccine-induced seropositivity in this surveillance could be a result of receiving fewer than 3 vaccine doses) it seems likely that the true vaccination coverage among women attending sexual health clinics is lower than in the general population. This could be lower still if some

natural infections with both HPV16/18 in this population were wrongly assigned as having vaccine-induced seropositivity (discussed further below in the limitations of this surveillance). Our surveillance also demonstrates lower vaccine-induced seropositivity among women born outside the UK, women from more deprived areas and women with a history of chlamydia diagnosis. A difference in uptake by ethnic group was especially clear although this seemed to be largely explained by differences in deprivation and country of birth. The suggestion that HPV vaccination uptake may be lower for women attending sexual health clinics could call for consideration of mop-up vaccination in this setting, albeit largely based at this point in time on evidence from the catch-up cohorts (i.e. offering the vaccine to women aged up to 17 years old attending sexual health clinics, particularly women born outside of the UK, women from more deprived areas, or possibly of black women or women from other ethnic groups where vaccine-induced seropositivity appeared to be lower). Similarly, this raises the importance of ensuring that these women participate in cervical screening in the future as unvaccinated women will be at higher risk for cervical cancer than vaccinated women.

Others have considered inequality of HPV vaccination coverage in the UK. Sacks et al compared self-reported HPV vaccination status for 2,247 females ages 13-19 attending sexual health services across England in 2011[141]. This study showed lower HPV vaccination coverage compared to the general population. Among those offered the vaccine, there was lower completion among black women, women not in education, employment or training, women living in London, smokers and those with an STI diagnosis. Bowyer et al compared self-collected HPV-vaccination status among 1,912 girls in school year 11 (aged 15-16 years old at the time of participation) who would have been offered routine HPV vaccine at 12-13 years old. The authors demonstrated 3-dose uptake to be lower among black girls, Asian girls and girls from other ethnic groups compared to white girls[144]. Two studies

compared HPV vaccination among women eligible for routine HPV vaccination using data obtained from Child Health Information Systems [145, 146]. Both studies showed higher vaccination uptake among white girls compared to other ethnic groups. One of these studies (n=2,817) showed lower uptake in more deprived areas[145] whereas the other study (n=14,282) showed little evidence of differences in vaccine uptake by deprivation[146]. Finally, an ecological study conducted at PHE, which compared published estimates of area-level HPV vaccination coverage, demonstrated lower coverage in more deprived areas for the older catch-up cohorts (women offered the vaccine at 16-18 years old)[82].

We conducted two analyses for the case-control study to consider the association between the bivalent vaccine and diagnoses of genital warts. The odds ratio calculated using cases and controls sampled using both exclusive and concurrent sampling (including 553 cases and 1,548 controls) could have over-estimated the vaccine-effectiveness. We therefore conducted a second, matched analysis including only cases and controls selected using concurrent sampling (281 cases and 644 controls). In the end, this distinction was less important as we found no evidence of an association between HPV vaccine-induced seropositivity and the odds (or rate) of genital warts diagnoses using either analysis. These results do not support the hypothesis, born from ecological analyses, which showed declines in diagnoses of genital warts among women in vaccinated age-groups [84, 159], and post-hoc analyses from a clinical trial which also suggested a moderately protective effect of the bivalent vaccine against HPV types 6 and 11 combined [74]. However, this is consistent with some other findings since the introduction of the HPV vaccination programme in England and other countries. A population-based study in the Czech Republic compared genital warts acquisition by self-reported vaccination status. This study showed no evidence of protection against genital warts from the bivalent vaccine[167]. In another study among 1,198 young STI clinic attenders in

the Netherlands, the prevalence ratio (PR) for anogenital warts comparing unvaccinated women with women vaccinated with at least one dose was 0.67 (95% CI; 0.22 to 2.07). In the same population, there was no evidence of any protection against HPV types 6 and 11 (adjusted PR 1.03; 95% CI 0.74 to 1.43)[98]. In post-vaccination surveillance of HPV DNA infection among 15,459 young sexually active women in England, there has been no evidence of a change in the prevalence of HPV 6/11 infection within the post-vaccination period (paper submitted for publication). We have previously discussed alternative explanations for the ecological reductions in genital warts but concluded that a moderate protective effect of the bivalent vaccine was the most plausible justification as the magnitude of other potential explanations were not sufficient to explain the observed declines[84, 159]. In light of our results presented in this paper, it seems more likely that there is either another unexplored change which caused this decline in genital warts at the population level, or that this is a result of more than one change (i.e. a combination of more than one of the previously explored explanations).

These data and analyses have some limitations. Firstly, a limitation of this study was the arbitrarily defined cut-offs for seropositivity for the L1 proteins of HPV16 and HPV18. The numerical MFI value used for cut-off definition was lower than what has been used in other studies using the same technology; this was based on a set of polystyrene beads with slightly diminished loading capacity. However, sensitivity analyses exploring different cut-offs (80 MFI and 120 MFI) made little change to the overall conclusions of this manuscript. Furthermore, A comparisons of GST-L1 multiplex serology with other serological methods has shown excellent correlation for both HPV16 and HPV18 with the gold-standard pseudovirion-based neutralization assay (PBNA; correlation coefficients of 0.95 and 0.93, respectively)[168, 169] and the assay has been utilized in large-scale HPV vaccine trials[170]. Secondly, our measure of vaccine-induced seropositivity is likely to have

been overestimated as we assumed all women with dual seropositivity had vaccine-induced seropositivity. Among a sample of lower risk women aged 15-20 years who were attending for routine microbiological and biochemical investigations prior to the introduction of HPV vaccination around 1.8% were seropositive for both HPV16 and HPV18[23]. In this population of women attending a sexual health clinic, this proportion can be expected to be higher which would have incorrectly inflated our estimates of vaccine-induced seropositivity. Another limitation is that the number of residual samples that were requested but not obtained was higher than expected (19% compared to 10% expected). As a consequence, our sample size was slightly lower than originally planned and our power to consider differences in vaccine-induced seropositivity between different ethnic groups was therefore lower. Finally, we adjusted the case-control analysis for the presence of certain STIs as a proxy for sexual behaviour but there may have been other differences in sexual behaviour which this adjustment did not address. In addition, past history of STIs in GUMCAD is limited as data collection started in 2008 and pseudo-anonymised patient records can only be linked within a particular clinic. Therefore, STI diagnoses prior to 2008 or recorded at different clinics were not identified.

We should also consider the appropriateness of our control selection. Eligible cases and controls in this surveillance all attended one of six sexual health clinics and had a blood sample taken for an HIV/syphilis test. However, it is plausible that there are some important differences between cases and controls. Firstly, cases were all diagnosed with genital warts and were likely to have experienced symptoms. Some controls may well have been attending for routine sexual health examinations and if this health seeking behaviour also meant that controls were more likely to be vaccinated then this could have introduced a potential bias and potential overestimation of vaccine effectiveness. Secondly, it’s possible that sexual risk could differ between cases and controls, a potential bias if women with genital warts

diagnoses are higher risk women who have lower vaccination uptake, as we have demonstrated here in our first analysis. Both of these biases would work towards showing an apparent protective effect against genital warts (i.e. OR<1 for the association of vaccination with genital warts). As we did not see a protective effect, these potential biases do not throw doubt on our finding of no protective effect. For this finding to be incorrect, we need to suppose a bias that erroneously increased the odds ratio towards the null. It is possible that residual negative confounding masked a protective effect of the vaccine, but we can think of no plausible alternative reason why cases would have higher uptake of vaccination than controls.

In conclusion, our surveillance does not support a moderate cross-protective effect of the bivalent vaccine against genital warts. Vaccine-induced seropositivity in this high-risk population is similar to the aggregate national data on 3-dose population coverage but lower than the 1-dose uptake. Together with the likelihood of overestimation of vaccine-induced seropositivity by our testing method, women attending sexual health clinics probably have a slightly lower HPV vaccination coverage overall, and in some sub-groups in particular.

Table 1: Age at which the bivalent HPV vaccination would have been first offered in the National HPV Immunisation Programme, by age and year of sample collection

Year of sample collection	Age				
	16 years old	17 years old	18 years old	19 years old	20 years old
2011	12-16 yrs n=218	14-17 yrs n=139	15-18 yrs n=209	16-18 yrs n=362	17-18 yrs n=332
2012	12-15 yrs n=147	12-16 yrs n=93	14-17 yrs n=188	15-18 yrs n=320	n=0[1]
2013	12-13 yrs n=101	12-15 yrs n=103	12-16 yrs n=207	14-17 yrs n=279	n=0[1]
2014	12-13 yrs n=83	12-13 yrs n=128	12-15 yrs n=203	12-16 yrs n=215	n=0[1]
2015	12-13 yrs n=89	12-13 yrs n=137	12-13 yrs n=168	12-15 yrs n=238	n=0[1]

Cells shaded red identify women who would have been offered vaccination routinely (at age 12-13 years old); cells shaded green identify women who would have been offered vaccination as part of the catch-up (at age 14-18 years old); cells shaded grey identify women who may have been offered the vaccine as part of the routine or catch-up programme

1: No residual serum specimens were requested from 20 year old women in 2012 to 2015

Table 2: Patient characteristics and estimated HPV vaccine-induced seropositivity

	All women		Routine vaccination cohorts[1]		Catch-up vaccination cohorts[1]	
	n (%)	Vaccine-induced seropositivity[2]; % (95% CI)	n (%)	Vaccine-induced seropositivity; % (95% CI)	n (%)	Vaccine-induced seropositivity; % (95% CI)
Age						
16 years old	638 (16.1%)	72.4% (68.6%, 75.9%)	273 (38.7%)	81.6% (76.2%, 85.9%)	0 (0%)	-
17 years old	600 (15.2%)	73.1% (69.1%, 76.8%)	265 (37.5%)	81.6% (76.2%, 86.0%)	139 (7.6%)	62.4% (52.8%, 71.2%)
18 years old	975 (24.6%)	68.3% (69.5%, 71.5%)	168 (23.8%)	74.5% (66.7%, 81.0%)	397 (21.7%)	56.7% (51.1%, 62.1%)
19 years old	1,414 (35.7%)	61.3% (58.5%, 64.2%)	0 (0%)	-	961 (52.5%)	54.4% (50.9%, 57.9%)
20 years old	332 (8.4%)	44.8% (38.8%, 50.9%)	0 (0%)	-	332 (18.2%)	44.8% (38.8%, 50.9%)
Ethnic group						
White	3,217 (82.6%)	68.0% (66.2%, 69.8%)	618 (90.5%)	80.0% (76.4%, 83.1%)	1416 (78.2%)	57.0% (54.0%, 59.9%)
Black	357 (9.2%)	49.5% (43.9%, 55.1%)	14 (2%)	85.2% (55.9%, 96.3%)	243 (13.4%)	43.6% (36.9%, 50.5%)
Asian	49 (1.3%)	65.9% (50.4%, 78.6%)	9 (1.3%)	88.4% (48.7%, 98.4%)	24 (1.3%)	46.5% (26.1%, 68.2%)
Mixed	239 (6.1%)	57.1% (50.2%, 63.9%)	30 (4.4%)	90.7% (69.8%, 97.6%)	116 (6.4%)	41.6% (32.2%, 51.6%)
Other[3]	33 (0.8%)	41.2% (25.1%, 59.5%)	12 (1.8%)	60.3% (30.0%, 84.3%)	11 (0.6%)	30.8% (10.3%, 63.2%)
Unknown	64		23		19	
Country of birth						
UK	3,504 (92%)	66.5% (64.8%, 68.2%)	590 (92.3%)	81.7% (78.1%, 84.7%)	1652 (91.4%)	55.6% (52.9%, 58.3%)
Outside of UK	306 (8%)	47.5% (41.4%, 53.6%)	49 (7.7%)	64.0% (49.0%, 76.7%)	156 (8.6%)	37.3% (29.3%, 45.9%)
Unknown	149		67		21	

Quintile of deprivation						
Q5 (least deprived)	854 (21.6%)	71.0% (67.6%, 74.2%)	168 (23.8%)	84.5% (78.8%, 89.5%)	361 (19.7%)	62.8% (57.2%, 68.0%)
Q4	698 (17.6%)	73.5% (69.6%, 77.0%)	136 (19.3%)	83.3% (75.5%, 89.0%)	306 (16.7%)	62.5% (56.0%, 68.7%)
Q3	648 (16.4%)	69.2% (65.0%, 73.1%)	137 (19.4%)	80.7% (72.7%, 86.8%)	283 (15.5%)	59.6% (52.9%, 66.1%)
Q2	586 (14.8%)	62.6% (58.2%, 66.9%)	96 (13.6%)	77.4% (67.6%, 84.9%)	265 (14.5%)	49.4% (42.7%, 56.1%)
Q1 (most deprived)	1,172 (29.6%)	55.5% (52.3%, 58.6%)	169 (23.9%)	73.3% (65.6%, 79.8%)	613 (33.5%)	43.8% (39.5%, 48.2%)
Unknown	1				1	
Herpes (current or previous attendance)						
No	3,769 (95.2%)	65.2% (63.5%, 66.8%)	676 (95.8%)	79.5% (76.0%, 82.5%)	1729 (94.5%)	53.2% (50.5%, 55.9%)
Yes	190 (4.8%)	66.5% (58.9%, 73.4%)	30 (4.2%)	89.0% (70.9%, 96.4%)	100 (5.5%)	62.7% (52.1%, 72.3%)
Gonorrhoea (current or previous attendance)						
No	3,807 (96.2%)	65.8% (64.1%, 67.4%)	689 (97.6%)	80.1% (76.7%, 83.0%)	1749 (95.6%)	54.2% (51.6%, 56.8%)
Yes	152 (3.8%)	53.4% (45.0%, 61.7%)	17 (2.4%)	73.1% (46.4%, 89.5%)	80 (4.4%)	45.3% (34.1%, 57.0%)
Chlamydia (current or previous attendance)						
No	3,286 (83%)	66.5% (64.7%, 68.3%)	608 (86.1%)	78.9% (75.2%, 82.1%)	1475 (80.6%)	56.0% (53.1%, 58.8%)
Yes	673 (17%)	59.5% (55.4%, 63.5%)	98 (13.9%)	86.1% (77.2%, 91.9%)	354 (19.4%)	45.2% (39.6%, 50.9%)
Genital warts[4] (current or previous attendance)						
No	3,235 (81.7%)	65.2% (63.6%, 66.9%)	632 (89.5%)	79.9% (76.6%, 82.9%)	1436 (78.5%)	53.8% (51.2%, 56.3%)
Yes	724 (18.3%)	67.5% (63.2%, 71.5%)	74 (10.5%)	71.5% (58.0%, 82.1%)	393 (21.5%)	56.3% (51.0%, 61.5%)

1: Vaccination cohorts determined from age and year at sample collection (see Table 1); routine vaccination cohorts include women offered the HPV vaccine at age 12-13 years; catch-up vaccination cohorts include women offered the HPV vaccine at age 14-18 years

2: estimates are weighted to account for the oversampling of specimens from women with genital warts

3: includes women categorised as "Chinese" or "any other ethnic group"

4: Women with a diagnosis of genital warts were oversampled for the nested case-control study

Table 3: Odds ratios for the association between bivalent vaccination status and diagnosis of genital warts

All cases (n=576) and controls (n=1,638)		Analysis restricted to concurrent sampling: cases (n=303) and matched controls (n=753)	
OR (95% CI) adjusted for matching variables[1]	OR (95% CI) adjusted for all variables[2]	Unadjusted OR (95% CI)	Adjusted[3] OR (95% CI)
n=2,101	n=2,101	n=925	n=925
1.08 (0.88, 1.34)	1.05 (0.84, 1.29)	1.05 (0.75, 1.46)	1.02 (0.72, 1.45)

1: adjusted for quarter, age and testing laboratory to account for matching

2: adjusted for quarter, age, testing laboratory, ethnic group, country of birth, quintile of IMD and diagnosis (past or present) with gonorrhoea, chlamydia or herpes

3: conditional logistic regression adjusted for ethnic group, country of birth, quintile of IMD and diagnosis (past or present) with gonorrhoea, chlamydia or herpes

Figure 1: Specimen collection and eligibility for different analyses

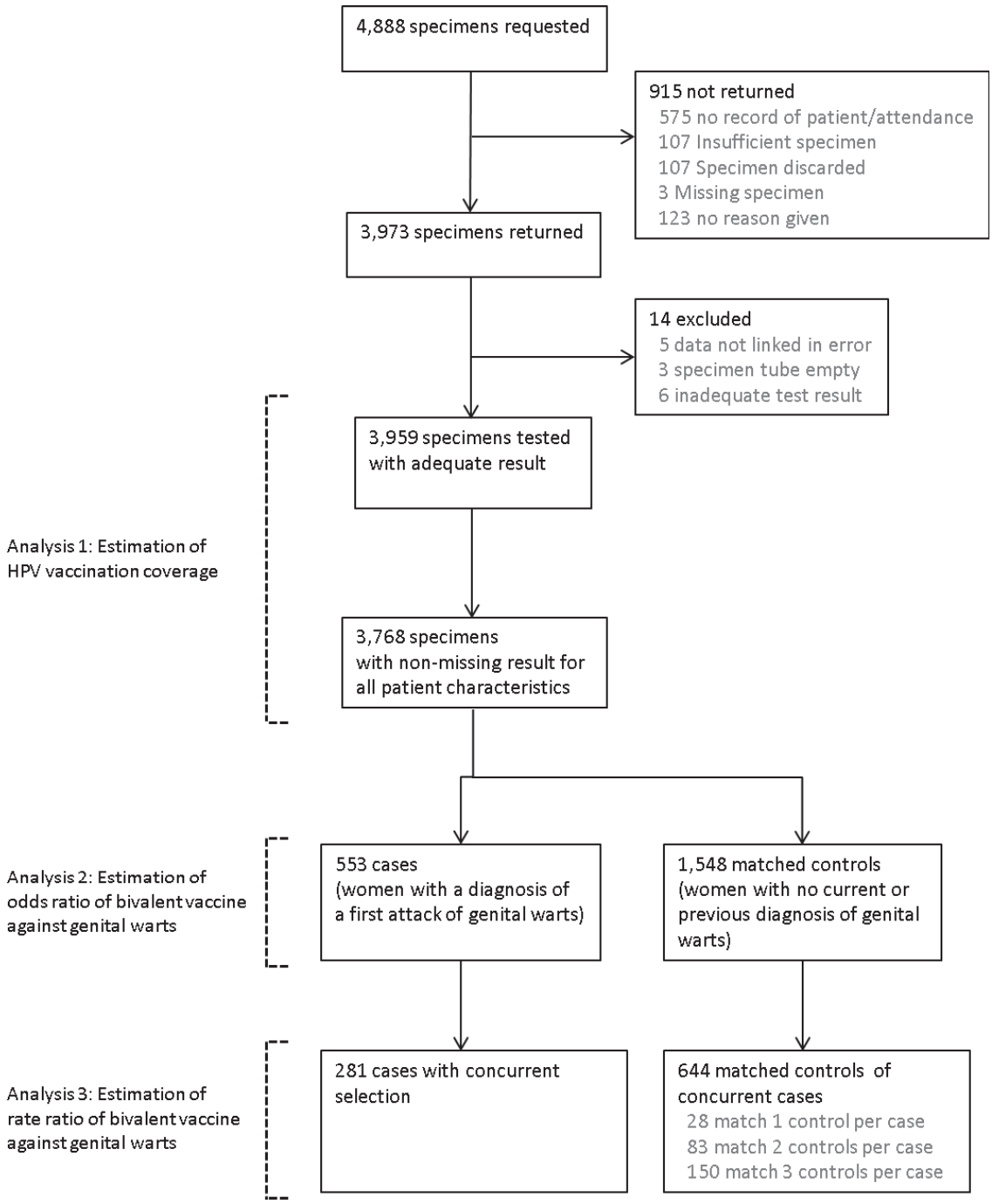
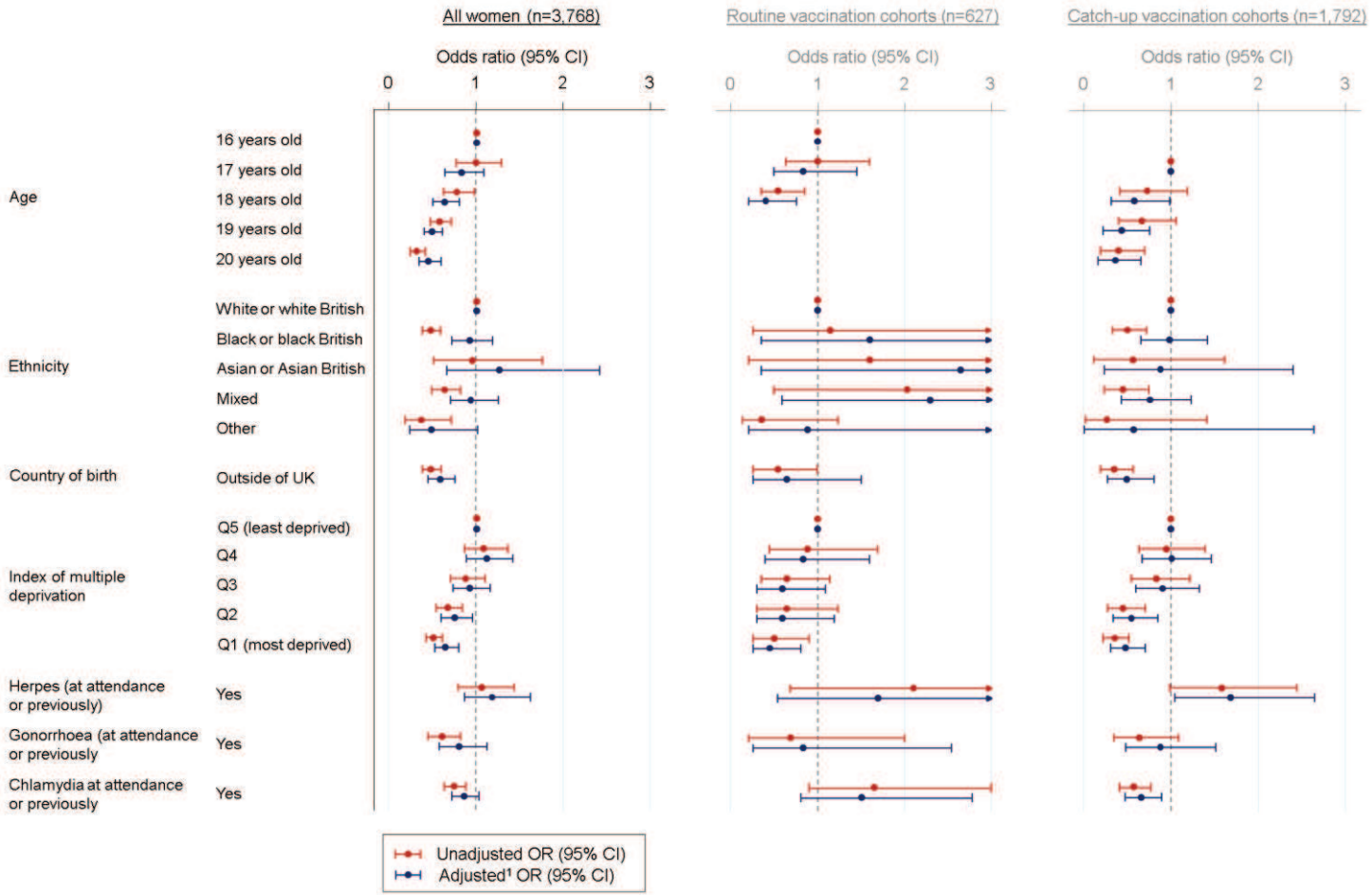


Figure 2: Adjusted and unadjusted odds ratio for vaccine-induced seropositivity, by patient characteristics



1: Adjusted for ethnicity; quintile of IMD; whether the patient had a concurrent or previous diagnosis of syphilis, gonorrhoea or chlamydia; country of birth (UK vs. outside of the UK); age at attendance and year of specimen collection

Supplementary Table 1: Adjusted and unadjusted odds ratio for vaccine-induced seropositivity, by patient characteristics

	All women		Routine vaccination cohorts[1]		Catch-up vaccination cohorts[1]	
	Unadjusted OR (95% CI)	Adjusted OR[2] (95% CI)	Unadjusted OR (95% CI)	Adjusted OR[2] (95% CI)	Unadjusted OR (95% CI)	Adjusted OR[2] (95% CI)
Age						
16 years old	1.0	1.0	1.0	1.0	-	-
17 years old	1.0 (0.8, 1.3)	0.8 (0.6, 1.1)	1.0 (0.6, 1.6)	0.8 (0.5, 1.4)	1.0	1.0
18 years old	0.8 (0.6, 1.0)	0.6 (0.5, 0.8)	0.5 (0.3, 0.9)	0.4 (0.2, 0.7)	0.8 (0.5, 1.2)	0.7 (0.4, 1)
19 years old	0.6 (0.5, 0.7)	0.5 (0.4, 0.6)	-	-	0.7 (0.5, 1.1)	0.5 (0.4, 0.8)
20 years old	0.3 (0.2, 0.4)	0.4 (0.3, 0.6)	-	-	0.5 (0.3, 0.8)	0.5 (0.3, 0.7)
Ethnic group						
White	1.0	1.0	1.0	1.0	1.0	1.0
Black	0.5 (0.4, 0.6)	0.9 (0.7, 1.2)	1.1 (0.3, 5.0)	1.6 (0.3, 7.5)	0.6 (0.4, 0.8)	1.0 (0.7, 1.4)
Asian	0.9 (0.5, 1.8)	1.3 (0.7, 2.4)	1.6 (0.2, 11.2)	2.6 (0.3, 20.7)	0.6 (0.3, 1.5)	0.9 (0.4, 2.2)
Mixed	0.6 (0.5, 0.8)	0.9 (0.7, 1.3)	2.0 (0.5, 8.0)	2.3 (0.6, 9.4)	0.5 (0.4, 0.8)	0.8 (0.5, 1.2)
Other[3]	0.4 (0.2, 0.7)	0.5 (0.2, 1.0)	0.4 (0.1, 1.2)	0.9 (0.2, 3.7)	0.4 (0.1, 1.3)	0.6 (0.2, 2.4)
Country of birth						
UK	1.0	1.0	1.0	1.0	1.0	1.0
Outside of UK	0.5 (0.4, 0.6)	0.6 (0.4, 0.8)	0.5 (0.3, 1.0)	0.6 (0.3, 1.5)	0.5 (0.3, 0.6)	0.6 (0.4, 0.8)
Quintile of deprivation						
Q5 (least deprived)	1.0	1.0	1.0	1.0	1.0	1.0
Q4	1.1 (0.9, 1.4)	1.1 (0.9, 1.4)	0.9 (0.4, 1.7)	0.8 (0.4, 1.6)	1.0 (0.7, 1.3)	1.0 (0.7, 1.4)
Q3	0.9 (0.7, 1.1)	0.9 (0.7, 1.2)	0.6 (0.3, 1.1)	0.6 (0.3, 1.1)	0.9 (0.6, 1.2)	0.9 (0.7, 1.3)
Q2	0.7 (0.5, 0.8)	0.8 (0.6, 1.0)	0.6 (0.3, 1.2)	0.6 (0.3, 1.2)	0.5 (0.4, 0.8)	0.6 (0.5, 0.9)
Q1 (most deprived)	0.5 (0.4, 0.6)	0.6 (0.5, 0.8)	0.5 (0.3, 0.9)	0.4 (0.2, 0.8)	0.5 (0.4, 0.6)	0.6 (0.4, 0.8)

Herpes (current or previous attendance)						
No	1.0	1.0	1.0	1.0	1.0	1.0
Yes	1.1 (0.8, 1.4)	1.2 (0.9, 1.6)	2.1 (0.7, 6.4)	1.7 (0.5, 5.4)	1.5 (1.0, 2.2)	1.6 (1.0, 2.4)
Gonorrhoea (current or previous attendance)						
No	1.0	1.0	1.0	1.0	1.0	1.0
Yes	0.6 (0.4, 0.8)	1.2 (0.9, 1.6)	0.7 (0.2, 2.0)	0.8 (0.3, 2.5)	0.7 (0.5, 1.1)	0.9 (0.6, 1.4)
Chlamydia (current or previous attendance)						
No	1.0	1.0	1.0	1.0	1.0	1.0
Yes	0.7 (0.6, 0.9)	0.9 (0.7, 1.0)	1.7 (0.9, 3.0)	1.5 (0.8, 2.8)	0.6 (0.5, 0.8)	0.7 (0.6, 0.9)

1: Vaccination cohorts determined from age and year at sample collection (see Table 1); routine vaccination cohorts include women offered the HPV vaccine at age 12-13 years; catch-up vaccination cohorts include women offered the HPV vaccine at age 14-18 years
2: Adjusted for ethnicity; quintile of IMD; whether the patient had a concurrent or previous diagnosis of syphilis, gonorrhoea or chlamydia; country of birth (UK vs. outside of the UK); age at attendance and year of specimen collection

3: includes women categorised as "Chinese" or "any other ethnic group"

8.5. Additional discussion of the serological surveillance

The above sections include the results from two serological surveillance studies (the SEU serosurveillance in Sections 8.2 and 8.3, and the serosurveillance among sexual health clinic attenders in Section 8.4). There were some limitations of the SEU serosurveillance, notably the assumption that the women included in this surveillance were representative of the general population. I discuss this along with other limitations in the paper and report presented in Sections 8.2 and 8.3. What follows below, are more specific issues relating to the serosurveillance among sexual health clinic attenders.

There were three key elements of the serosurveillance among sexual health clinic attenders which were not conducted as originally planned. The first was the change from exclusive sampling to concurrent sampling of cases and controls. The rationale and implications for this change was described in the Section 7.5.4. I presented results using each sampling technique in Section 8.4 and, as highlighted in the paper, the results using both approaches were very similar. I also discuss this in the paper (Section 8.4) so I do not discuss further here. The second and third changes relate to the HPV assay and were outside of my control. The change from using the VLP-based ELISA assay to the Luminex-based GST-T1 multiplex serology assay (described in Section 7.4.5) was unfortunate as this delayed testing. However, the more serious issue was that, due to a problem encountered by the laboratory with the testing, the results from this assay were not able to provide a quantifiable measure of antibody concentration. This was described in detail in Section 7.4.6 and meant that the only approach available for this analysis was to assume all women with dual seropositivity for HPV16 and HPV18 had vaccine-induced seropositivity, rather than to delineate women with possible or probable vaccine-induced seropositivity based on their antibody concentrations, as described in Section 7.3.3. The implications of the potential misclassification of vaccination

status are described below. I also provide a more detailed discussion of other limitations of this surveillance and potential biases for the nested case-control study.

8.5.1. Potential misclassification of vaccination status in the serosurveillance among sexual health clinic attenders

As described above, I assumed all women with dual seropositivity for HPV16 and HPV18 had vaccine-induced seropositivity for the serosurveillance among sexual health clinic attenders in Section 8.4. However, serological data from the PHE SEU which were tested prior to the introduction of HPV vaccination demonstrated that 1.8% (95% confidence internal: 0.9%-3.4%) of 15-19 year old women were seropositive for both HPV16 and HPV18 due to natural infection. The women included in the serosurveillance among sexual health clinic attenders in this thesis are likely to have an even higher risk of dual seropositivity following natural infection. Therefore, by assuming that all women seropositive for HPV16 and HPV18 had vaccine-induced seropositivity, I will have likely overestimated the true HPV vaccination coverage in this population.

In Table 8.1, the estimated extent of potential overestimation of vaccine-induced seropositivity is presented. I considered varying levels of natural infection of 0% (i.e. no overestimation), 1.8% (the pre-vaccination prevalence of natural infection in the SEU data), 3.0%, 4.5% and 6.0%. I also considered, as suggested in Section 8.4, that women at higher risk of HPV infection could be less likely to be vaccinated, with relative risks of natural infection compared to unvaccinated women of 1.0 (i.e. assuming natural infection is not associated with vaccination), 0.9, 0.8 and 0.7. Estimated true vaccination coverage was calculated as follows:

$$Vaccine\ coverage_{neg} = \frac{Observed\ vaccine\ coverage - \ p_{pos}}{1 - \ p_{pos}}$$

$$Vaccine\ coverage_{pos} = RR * \frac{Observed\ vaccination\ coverage - \ p_{pos}}{1 - \ p_{pos}}$$

hence;

$$True\ vaccine\ coverage = Vaccine\ coverage_{neg} * (1 - p_{pos}) + Vaccine\ coverage_{pos} * (p_{pos})$$

Where;

$Observed\ vaccine\ coverage$ is 65.2% in the surveillance included in this thesis (i.e. the proportion with vaccine-induced seropositivity)

p_{pos} is the proportion of women who would have natural seropositivity for HPV16 and HPV18 in an unvaccinated population

$Vaccination\ coverage_{neg}$ is the vaccination coverage in women without natural seropositivity for HPV16 and HPV18

$Vaccination\ coverage_{pos}$ is the vaccination coverage in women with natural seropositivity for HPV16 and HPV18

RR is the relative risk of HPV vaccination in women with natural seropositivity compared to women without natural seropositivity

In the most extreme scenario, with 6% of the population having dual seropositivity from natural infection and a 30% lower risk of being vaccinated in women with natural infection, the estimated true vaccination coverage was 61.8% compared to the observed coverage of 65.2%. This suggests that even in a worst-case scenario, the overestimation of true vaccination coverage in Section 8.4 is less than 5%.

As well as considering overestimation of vaccination coverage, I also considered the impact of misclassification of vaccination status on the results of the nested case-control study. If this was non-differential misclassification of vaccination status (i.e. inaccurately assigning a woman as vaccinated was unrelated to having genital warts) then this bias would likely have resulted in an underestimate of the vaccine effectiveness. However, it is likely that those with inaccurate recording were from

higher-risk populations with a higher risk of dual seropositivity from natural infection. If these women were also more likely to be diagnosed with genital warts, then this differential misclassification bias would tend to overestimate any protective effect of the bivalent vaccine against genital warts and thus would not explain the lack of protective effectiveness which was seen.

The results of the above section suggest that this misclassification is unlikely to have had a large impact on the results of the serosurveillance among sexual health clinic attenders presented in Section 8.4. However, it is a limitation of this analysis that I was not able to use a similar approach as the one I developed for the SEU serosurveillance to ascertain probable vaccine-induced seropositivity based on antibody concentrations (see Section 7.3.3). As described in Section 7.4.6, the reason that antibody concentrations could not be assessed for the serosurveillance among sexual health clinic attenders was due to an unexpected degradation of the polystyrene beads used in the assay. Therefore, my colleagues at PHE and I are currently considering options to have these residual specimens re-tested to address this limitation. Future re-testing of specimens would enable updating of the analysis presented in Section 8.4.

Table 8.1: Potential misclassification of vaccination status for serosurveillance among sexual health clinic attenders. Numbers are estimated vaccination coverage in different scenarios

Relative risk of being vaccinated in women with natural dual seropositivity[1]	Proportion of women with natural seropositivity for HPV16 and HPV18 in an unvaccinated population				
	0.0%	1.8%	3.0%	4.5%	6.0%
1.0	65.2%	64.6%	64.1%	63.6%	63.0%
0.9	65.2%	64.4%	63.9%	63.3%	62.6%
0.8	65.2%	64.3%	63.7%	63.0%	62.2%
0.7	65.2%	64.2%	63.5%	62.7%	61.8%

1: The relative risk of HPV vaccination in women with natural seropositivity compared to women without natural seropositivity

8.5.2. *Potential bias due to missing data and unmeasured confounding in the serosurveillance among sexual health clinic attenders*

There were missing data for ethnicity, IMD and/or country of birth for around 5% of women included in the serosurveillance among sexual health clinic attenders. In Table 8.2, I compared the patient characteristics for all women with the patient characteristics for women with and without missing data. IMD was only missing for one woman (who also had missing data on both ethnicity and country of birth). Women with missing data for ethnicity were far more likely to have missing data for country of birth compared to women with non-missing ethnicity data (34.4% vs. 3.3% respectively) and vice versa. However, reassuringly patient characteristics were similar for all women and for women with no missing data (the latter group being those used for regression analyses).

Table 8.2: Patient characteristics among all women, women with no missing data and women with missing data for at least one variable

	All women (n=3,959)	Women with no missing data (n=3,768)	Women with some missing data (n=191)[1]
	n (%)	n (%)	n (%)
Age			
16 years old	638 (16.1%)	614 (16.3%)	24 (12.6%)
17 years old	600 (15.2%)	549 (14.6%)	51 (26.7%)
18 years old	975 (24.6%)	930 (24.7%)	45 (23.6%)
19 years old	1414 (35.7%)	1348 (35.8%)	66 (34.6%)
20 years old	332 (8.4%)	327 (8.7%)	5 (2.6%)
Ethnic group			
White	3217 (82.6%)	3112 (82.6%)	105 (82.7%)
Black	357 (9.2%)	347 (9.2%)	10 (7.9%)
Asian	49 (1.3%)	44 (1.2%)	masked[5]
Mixed	239 (6.1%)	233 (6.2%)	6 (4.7%)
Other[3]	33 (0.8%)	32 (0.8%)	masked[5]
Country of birth			
UK	3504 (92%)	3476 (92.3%)	28 (66.7%)
Outside of UK	306 (8%)	292 (7.7%)	14 (33.3%)
Quintile of deprivation			
Q1 (most deprived)	1172 (29.6%)	1112 (29.5%)	60 (31.6%)

Q2	586 (14.8%)	555 (14.7%)	31 (16.3%)
Q3	648 (16.4%)	623 (16.5%)	25 (13.2%)
Q4	698 (17.6%)	651 (17.3%)	47 (24.7%)
Q5 (least deprived)	854 (21.6%)	827 (21.9%)	27 (14.2%)
Herpes (diagnosed at current or previous attendance)			
No	3769 (95.2%)	3587 (95.2%)	182 (95.3%)
Yes	190 (4.8%)	181 (4.8%)	9 (4.7%)
Gonorrhoea (diagnosed at current or previous attendance)			
No	3807 (96.2%)	3623 (96.2%)	184 (96.3%)
Yes	152 (3.8%)	145 (3.8%)	7 (3.7%)
Chlamydia (diagnosed at current or previous attendance)			
No	3286 (83%)	3116 (82.7%)	170 (89%)
Yes	673 (17%)	652 (17.3%)	21 (11%)
Genital warts[4] (diagnosed at current or previous attendance)			
No	3235 (81.7%)	3070 (81.5%)	165 (86.4%)
Yes	724 (18.3%)	698 (18.5%)	26 (13.6%)

1: Women missing data for ethnicity (n=64), IMD (n=1) or country of birth (n=149)

2: estimates are weighted to account for the oversampling of specimens from women with genital warts

3: includes women categorised as "Chinese" or "any other ethnic group"

4: Women with a diagnosis of genital warts were oversampled for the nested case-control study

5: In accordance with PHE data sharing policy, cells with values between 1 and 4 inclusive were masked. If masked cells could be deduced from values of other cells then the next smallest cell was also masked

An additional consideration is the potential effect of unmeasured sexual risk behaviour on the results of the serosurveillance among sexual health clinic attenders. I partially controlled for differences in sexual behaviour by adjusting for the presence of sexually transmitted infections (either at the current or at previous attendances) as a proxy. However, similarly to the analyses in Chapter 6, there are likely to have been other differences in sexual behaviour which were not addressed by adjusting for these STIs. A further complication in this surveillance is the potential underestimation of previous history of STIs using GUMCAD. GUMCAD data contain a pseudo-anonymised patient identifier to allow patient records within the same clinic to be linked to previous visits and STI diagnoses. However, no information on previous diagnoses or attendances made at other clinics is available. Furthermore,

GUMCAD data recording only started in 2008, so attendances and diagnoses before this date are not recorded. If the previous history of STIs were underestimated then this would mean there was further potential for residual confounding due to unmeasured differences in sexual behaviour. In this analysis, misclassification of the previous history of STIs could have resulted in residual confounding of both the analysis of variations in vaccine-induced seropositivity by patient characteristics and the estimation of vaccine effectiveness against genital warts. The effect of misclassification of a confounder varies according to whether the misclassification is non-differential or differential. In general, non-differential misclassification attenuates the adjustment of the confounder and the direction of the bias would depend on the direction of this confounding. The direction and magnitude of potential bias due to non-differential misclassification is harder to predict. For these analyses, it is difficult to know the extent to which unmeasured or underestimated sexual risk behaviour could have biased the reported results as there are limited data on the extent to which patients move between different sexual health clinics and whether this varies by patient characteristic.

8.5.3. Selection of controls for nested case-control study

There are further considerations about the appropriateness of the controls selected for the nested case-control study to investigate the potential protective effect of the bivalent vaccine against genital warts. Firstly, there is the possibility that controls may have been previously diagnosed with genital warts but that this was unrecorded. As described in the previous section, GUMCAD data are only able to identify previous STIs diagnosed at the same sexual health clinic since 2008. Controls could therefore have been previously diagnosed with genital warts and hence, according to the sampling described in Section 7.5.4, would not have been an eligible control for this case-control study. If controls who had an unrecorded previous diagnosis of genital warts were less likely to be vaccinated than correctly

classified controls (in line with the hypothesis that vaccination protects against warts) then this could have underestimated the vaccine effectiveness estimates. Conversely, if controls with a previous diagnosis of genital warts were more likely to be vaccinated than other controls (for example, if a previous diagnosis of warts prompted these women to get vaccinated) then their inclusion would have overestimated the vaccine effectiveness. As in the previous section, it is difficult to quantify this potential effect as there are limited data on the proportion of women who attend multiple sexual health clinics (and who therefore could have been diagnosed elsewhere prior to their attendance). Given that the results presented in the paper in Section 8.4, provided no evidence that the vaccine protected against genital warts, the former scenario (underestimation of vaccine effectiveness) is more pertinent. However, it seems unlikely that this could have entirely explained the lack of vaccine effectiveness found, as it would necessitate a large proportion of controls to have a previous history of genital warts and for these women to have similar odds of vaccination as the cases.

Secondly, as previously described in Section 7.5.2, the vast majority of women who present to health services with genital warts will attend a sexual health clinic[156] although there is limited information on the proportion of women with genital warts who do not attend any health services. A suitable control should be sampled from the population that gave rise to the cases (i.e. sexual health clinic attenders) and sampled independently of their exposure status (i.e. HPV vaccination). If these two principles are not met, this can lead to selection bias. Eligible women in this surveillance attended a sexual health clinic and had a blood sample taken for an HIV/syphilis test. Therefore, the first of these principles seems appropriate as controls attended the same sexual health clinic as the cases. The second of the above principles is less clear; cases with genital warts will almost certainly have been aware of their infection and may well have been attending the sexual health

clinic specifically for this purpose. Conversely, controls may have attended for other reasons; some controls may have attended for a symptomatic STI (other than genital warts); others may have been asymptomatic (regardless of whether they had another STI) and therefore may have attended due to a potential exposure to an STI or simply for a general sexual health check. If the latter women's health seeking behaviour extended to vaccination then the selected controls could have had a higher uptake of HPV vaccine than the background population, and this could have resulted in an overestimate of vaccine effectiveness. Again, given that the results in Section 8.4 did not demonstrate a protective effect of the bivalent vaccine against genital warts, overestimation of vaccine effectiveness is less of a concern here.

This concludes the findings of this thesis, including both the surveillance of HPV DNA infection and the serological surveillance. In the following chapter (Chapter 9), I summarise the overall findings from these studies and what these add to prior knowledge. I also discuss the overall strengths and limitations of the data sources used and how the studies were conducted. Finally, I consider the implications of the findings for prevention of HPV and for future research.

Chapter 9: Discussion

9.1. What this PhD adds to prior knowledge

I review below what was known prior to the start of this PhD and what the results of this PhD add to current understanding (either within England specifically or within the wider context).

9.1.1. Post-vaccination HPV infection surveillance (Chapters 3 to 6, Research questions 1 to 3)

What was already known?

The clinical trials for the bivalent vaccine demonstrated a very high prophylactic efficacy against HPV infection with vaccine types as well as some evidence of vaccine efficacy against some closely related high-risk HPV types, although the latter was less consistent across studies. However, the results from these randomised controlled trials told us little about the potential impact of national vaccination in a population setting which will differ for many reasons; (i) vaccinated women may not receive the vaccine according to the recommended timing, (ii) in a non-randomised setting there may be some inequalities in vaccination uptake which could affect the population-level impact, (iii) in population settings, particularly in countries with high vaccine coverage, there may be a herd protection effect in addition to the direct effect from vaccination, and (iv) there is potential for type replacement (i.e. non-vaccine types becoming more common in vaccinated populations). This latter effect was not seen in clinical trials although many trials were limited in population size and/or duration of follow-up which would limit the ability to detect changes in the prevalence of less common HPV types over time. It is important for population level surveillance to monitor infection with non-vaccine types to either quantify type-replacement if it does exist, or, to reinforce confidence in the vaccine and national programme if there is no evidence of type-replacement.

The UK introduced a national HPV vaccination programme for females from September 2008. Other countries introduced HPV vaccination with a similar timeframe to the UK and some of these countries vaccinated in similar settings and achieved similar vaccination coverage to the UK. As such, relevant information on the evaluation of HPV vaccination has not necessarily been restricted to data from the UK but also elsewhere in the world. Some early results of post-vaccination surveillance had been published at the start of this PhD. In the USA, there was some early evidence demonstrating declines in the vaccine-type HPV prevalence[87, 88, 171]. However, the vaccination coverage in the USA was much lower than in England hence these results were less relevant for our population. Australia had a more similar setting to the UK with vaccination coverage closer to coverage in England for routinely vaccinated women. The first data considering the impact of HPV vaccination on vaccine HPV types in Australia were published in October 2012[172]. This interim analysis of samples from 404 women demonstrated a substantial decrease in vaccine-targeted genotypes. Both the USA and Australia introduced the quadrivalent vaccine to their national programmes. In England, we published the first results of national HPV surveillance conducted by PHE in July 2013, the same month that this PhD started. This was the first evidence of reductions in the prevalence of HPV16/18 infection following introduction of a national bivalent vaccination programme.

What this thesis adds?

The national surveillance of type-specific HPV prevalence in England has added to the evidence of substantial reductions in HPV vaccine-type prevalence following introduction of a national vaccination programme (Chapter 6). The analyses comparing HPV vaccine-type prevalence among women with a known vaccination status in Section 6.3 suggest that these declines were due to direct protection of the vaccine (i.e. high vaccine effectiveness) and indirect herd protection. Because many

countries are conducting similar surveillance in their own countries this PhD has been well timed to add to international data as well as providing necessarily specific results to evaluate the programme in England. One notable difference of the UK national vaccination programme compared to other countries is that it was the first country to exclusively use the bivalent HPV vaccine in its national programme. Although the vaccine used was changed to the quadrivalent vaccine in 2012, there are nine birth cohorts of vaccinated women who were vaccinated with the bivalent vaccine and this offers an opportunity to monitor the impact of this vaccine on HPV infection and early disease outcomes. This is of particular interest when considering cross-protection as results from clinical trials suggested some differences in vaccine efficacy against non-vaccine types between the two vaccines[76]. The most recent analysis of national surveillance in England presented in the paper in Section 6.3 demonstrate greater declines in HPV31, 33 and 45 infection than those seen in countries introducing the quadrivalent vaccine. This is consistent with recent evidence from Scotland which has also shown substantial cross-protection[95].

This thesis also includes results from an international systematic review that examined the evidence for changes in non-vaccine types following HPV vaccine introduction. Combining the data using meta-analysis allowed exploration of changes in rarer non-vaccine types which could not be done in individual surveillance studies with limited sample sizes. This was the first meta-analysis to investigate changes in individual non-vaccine types and it did not provide any clear evidence for type replacement; an important finding to give reassurance that the vaccine is not only reducing vaccine-types but that vaccination is not leading to large increases in HPV infections with other high-risk types. This was also supported by updated results from the post-vaccination HPV infection surveillance in England which showed a relatively stable prevalence of non-vaccine and non-cross-protective high-risk types in the post-vaccination period (Chapter 6).

9.1.2. Immune response to the vaccine (Chapter 7 and 8, Research question 4)

What was already known?

Vaccination coverage in England was (and still is) reported nationally using local data. At the start of this PhD, 3-dose vaccine coverage for the routine cohorts was consistently reported to be above 80% but coverage for the catch-up cohorts was more variable (70.8% and 75.7% for younger catch-up cohorts largely vaccinated at schools; 38.9%, 47.4% and 48.1% for older catch-up cohorts vaccinated in different education and primary care settings). These national coverage data were stratified by birth cohort and local area but not by any other factors (e.g. ethnicity). If there were inequalities in delivering the vaccine then this could potentially affect the level of herd protection, leaving some population subgroups at a higher risk of subsequent HPV-related disease. Other studies have considered inequities of vaccination uptake but most were restricted to one local area and all relied on either self-collected vaccination status or CHIS data[82, 141, 144-146]. Therefore, there were no nationally representative and technically robust data to address whether there was different vaccine coverage in different sup-populations in England.

What this thesis adds?

The two serosurveillance studies included in this thesis are the first to estimate vaccine coverage in England using serological data. The data for the first of these two analyses were from a population which can be considered to approximate the general population (Sections 8.2 and 8.3) which is an additional strength of this analysis. These data confirm high coverage and immune response in younger women but an important finding that the proportion of women with an immune response in the older vaccination cohorts was slightly higher than national data suggests. This could be due to higher vaccine coverage which was not reported by local areas or a higher immune response in the partially vaccinated at older ages. A

more likely explanation is that this is due to a combination of both of these factors. Whilst it was anticipated that there may be some under-reporting in older catch-up cohorts, largely vaccinated outside of schools, the results from this surveillance are the first evidence of this. The analyses of these data also demonstrated that although there was some waning of antibody concentrations over time since vaccination, these remained far higher than the immune response following natural infection up to five years post-vaccination.

I also looked at immune response in a higher-risk population attending sexual health clinics to estimate vaccine uptake in this group and I compared uptake within subgroups of this high-risk population to identify if there were any inequalities in vaccine uptake (Section 8.4). This is not unique in itself, as one questionnaire-based study previously had considered HPV vaccination uptake in sexual health settings in England[141]. However, the analyses in this thesis do represent the only UK study to use serological data to monitor HPV immune response following vaccination. A relatively high proportion of women in this surveillance had an immune response to the vaccine types which was comparable to the proportion receiving 3-doses in the nationally published data. This surveillance also demonstrated lower vaccination uptake among women born outside of the UK, women from more deprived areas and women with a history of chlamydia diagnosis. There was lower uptake in some ethnic groups (black, mixed and other ethnicity) although this was less clear after adjustment for quintile of deprivation (IMD) and country of birth.

9.1.3. Does the bivalent vaccine protect against genital warts? (Chapter 7 and 8, Research question 5)

What was already known?

A post-hoc analysis of the PATRICIA clinical trial examined vaccine efficacy against low-risk HPV types[74]. These analyses showed a vaccine efficacy against low risk

types HPV6/11 of 34.5% (95% CI; 11.3-51.8). The evaluation of whether the bivalent vaccine had any impact on diagnoses of genital warts in a population setting could be uniquely investigated in England for two reasons; (i) the UK was one of very few countries who adopted exclusively the bivalent vaccine, and (ii) England have data on all attendances and diagnoses of all individuals attending sexual health clinics in England (where an estimated 95% of GW diagnoses are seen[156]). Ecological analysis of trends in genital warts diagnoses in England over time had suggested an association between increasing HPV vaccination coverage and modest declines in the diagnoses of genital warts in females[84], supporting the results from the post-hoc analysis of the PATRICIA trials.

What this thesis adds?

The analyses of the case-control study demonstrated no evidence of a protective effect of the bivalent HPV vaccine against acquisition of genital warts. Previous investigation of this research question at PHE had relied on ecological data. The results of this case-control study are less affected by other potential changes over time in sexual behaviour and service delivery at sexual health clinics and provide more reliable evidence. Whilst these results are inconsistent with the ecological findings in the UK (Sections 7.5.1) they are consistent with our findings of no changes in HPV6/11 prevalence within the post-vaccination period (Section 6.3).

9.2. Overall strengths and limitations of this PhD

In this thesis, I have presented results from different surveillance and epidemiological studies to consider several research questions. I have addressed specific limitations for each separate study and surveillance activity in Chapters 3, 6 and 8. One common factor throughout all the research conducted is the use of (i) routinely collected data, and (ii) residual specimens collected originally for other purposes. Such approaches come with their own unique strengths and limitations

which I explore below, along with some of the other overall strengths and limitations of this thesis.

9.2.1. Opportunistic use residual specimens and routinely collected data

Strengths: Many of the surveillance activities included in this thesis made use of routinely collected data which were linked prior to anonymisation and testing of residual specimens taken for other purposes. Data sources used in this thesis included the NCSP dataset, CTAD dataset, SEU dataset, GUMCAD and some additional linkage to Child Health Information Systems, general practices and the Office for National Statistics population data. The use of routinely collected data is relatively quick and cheap compared to primary data collection and allowed us to make use of the strengths of each different data source. Patient data could be used to determine surveillance eligibility. In addition, data on patient characteristics could be incorporated into multivariable models to control for potential confounding. Similarly, the use of residual samples allows collection of a large number of samples within a fairly short time frame and is relatively quick and cheap to establish. Furthermore, as the use of residual samples for surveillance to monitor the impact of national vaccination programmes falls under PHE's remit of public health monitoring, individual patient consent is not required. This provides the additional strength that, although the populations sampled may not be fully representative of the entire target population, there is less potential for the selection bias introduced by patient refusals.

Limitations: In using residual samples for HPV DNA and serology testing, there are some limitations. Firstly, it is necessary to identify a suitable sample type. For example, HPV DNA testing of urine is known to have a lower sensitivity for HPV detection in females than cervical specimens or vulva-vaginal swabs specimens. We also require a residual sample which has been stored correctly and has sufficient volume for the relevant testing platform. Using residual samples, we may

expect a higher proportion of inadequate samples due to insufficient volume or degradation of the sample collected outside of optimal conditions. Secondly, it is important to identify a suitable population. To determine changes in the prevalence HPV infection due to vaccination as soon as possible, it is necessary to identify a population of young women having suitable samples taken for other purposes. Collection of genital samples in young women restricted the choice to higher-risk populations of women attending sexual health services to be screened for sexually transmitted infection. I discuss below the strengths and limitations of conducting surveillance in high-risk populations (Section 9.2.3). Another limitation is that, within these populations, these are women with health seeking behaviour and those with even higher risk may not attend such settings.

There are also limitations using routinely collected data. Patient data are restricted to what is collected in the routine datasets so data could not be tailored to answer particular questions. For all surveillance activities included in this surveillance there are potential confounders which could not be adjusted for as the data were not available. This availability often changes as routine data collection is adapted over time so is sometimes not consistent within the surveillance. This was a limitation with the analysis of data from women attending for chlamydia screening with the change from the NCSP dataset (which collected data on sexual behaviour) to CTAD (which had no data on sexual behaviour). Another limitation is the often high proportion of missing data for data fields which are not mandatory. For example, ethnicity was missing for a considerable proportion of specimens and therefore could not be included in the analysis of the HPV infection surveillance (Chapter 6). Finally, as described in Section 5.1.1, there was no single source of routinely collected data which held individual-level HPV vaccination status data. This was a limitation for all of the surveillance activities included in this thesis. Firstly, for the HPV DNA surveillance (Chapter 6), this restricted the main analysis to considering

individual level outcomes (i.e. HPV prevalence) but ecological level exposure. Linkage to CHIS records was only possible for a relatively small proportion of women included in this surveillance which limited the ability to directly compare HPV prevalence in vaccinated and unvaccinated women (this is discussed further in the following section). Secondly, for the serological surveillance (Chapter 8), I determined probable vaccine status based on antibody concentrations for HPV16 and HPV18. Whilst this appeared to provide a robust proxy, I was unable to formally compare immune responses in known vaccinated vs. known unvaccinated women to validate this approach.

9.2.2. Collection of HPV vaccination status data

Strengths: Comparing HPV prevalence between the pre-vaccination and post-vaccination period or over time within the post-vaccination period provides important evidence of the population-level impact of HPV vaccination. However, this does not provide an estimate of the direct effect in vaccinated women compared to unvaccinated women (i.e. vaccine effectiveness). Collection of HPV vaccination status had been proposed prior to the start of this PhD but, due to the complications of data collection, the first data were not collected until 2014, as described in this thesis. Analyses of this data have not only allowed estimation of vaccine effectiveness in England but also allow estimation of the effect of herd protection among unvaccinated women.

Limitations: Other countries have national registers which collect and store HPV vaccination records for all women (for example, Scotland have a national vaccination register which can be linked using individuals' Community Health Index (CHI) number). In England, there is no national vaccination register; data are collected on different systems depending on where vaccination took place and these are collated only at a local level. I have described these limitations in detail in Chapter 5. The lack of a national database for vaccination records has meant that in

the final analysis of the HPV DNA surveillance, vaccination status data were only available for 21% of women eligible to receive the vaccine. As touched upon in the previous section, this affected my ability to estimate accurately the direct effect of the HPV vaccine.

9.2.3. Monitoring the impact of HPV vaccination in high-risk populations

Strengths: Serological surveillance in this thesis was conducted in both a high-risk population of women attending sexual health clinics and a more representative population; the appropriateness of the latter assumption for women having blood taken was discussed in Section 8.2. This allowed direct comparison of HPV immune response in the two populations. For the HPV DNA surveillance, women attending for chlamydia screening have a higher risk of chlamydia infection and hence likely also have a higher risk of HPV infection. This increases the power to look at changes in vaccine and related HPV types sooner than would be possible in lower risk populations. It is also encouraging that an impact on HPV infection is being seen among women at higher risk for HPV infection (and therefore subsequent HPV related disease). This reassures that there is not an inequality in vaccination of higher risk women.

Limitations: It is likely that if reductions in the prevalence of HPV infection are seen in high-risk populations then there will be reductions in the lower-risk populations. However, restricting analyses to higher risk women could potentially limit the representativeness of results of impact on infection and HPV vaccination uptake for the general population.

9.3. Implications for primary prevention of HPV and related diseases

9.3.1. Continuation of HPV vaccination of females

The main aim of the HPV vaccination programme is to reduce the incidence of cervical cancer. When the vaccine was introduced in 2008 for 12-18 year olds, dramatic declines in cervical cancers were not expected for several years given the peak age of cancers in England at around 25-29 years old[37]. Therefore, to wait for cancer registration data to demonstrate whether the vaccine is having an impact on HPV infection or disease would take at least 10 years since introduction to see the early impact of the programme, and even longer to see substantial declines. To wait 10 years whilst offering vaccination to all eligible females (approximately 250,000 routinely vaccinated women per year in England) before any data on the population level impact of the vaccine would clearly be unacceptable given the costs of vaccination. HPV vaccines are expensive compared to many other vaccines; although in England the price paid for the vaccine is kept confidential, in other high-income countries the HPV vaccines is one of the most expensive vaccines included in their national programmes[173]. By examining changes in HPV infection in younger women, this thesis provides earlier reassurance that there will very likely be a substantial reduction in the incidence of cervical disease and cervical cancer in England as vaccinated women reach the screening age and peak age of cervical cancer incidence. One concern of course is that the reductions in vaccine types and other closely-related HPV types may lead to other types becoming more common. This was seen following vaccination for pneumococcal infection[174]. Type replacement is considered less plausible for HPV due to the lower genetic mutation rate, but it is still important to remain vigilant for potential increases in non-vaccine types. Data from both the systematic review (Chapter 3) and considering changes within the post-vaccination period (Section 6.3) are consistent with there being no

clear evidence of type-replacement to date although ongoing monitoring of this is needed.

In light of all the positive evidence in this PhD, and from similar work conducted elsewhere in the world, the evidence is clear that an HPV vaccination programme should continue in England. I cover below how the results from this thesis may inform potential changes to how HPV vaccination is delivered.

9.3.2. Targeting groups with lower vaccination coverage

As described above, this thesis has shown some inequities in HPV vaccination uptake in some subgroups. This is of particular concern if women with lower HPV vaccination uptake are also women with lower cervical screening uptake as this will only widen this health inequality. If the HPV vaccine only provided a direct protection against HPV then these inequities would mean that an 80% reduction in HPV16/18 infections in the screened population may not necessarily transfer to an 80% reduction in HPV16/18 related cervical cancer. However, with the indirect effects of herd protection there is likely to be an impact on infection and disease among unvaccinated women, as has been demonstrated in this thesis. It is also very reassuring that we are seeing substantial declines in HPV vaccine types and closely related types in a higher risk population of women attending for chlamydia screening (Section 6.3). However, herd protection will be affected by the level of sexual mixing between these population subgroups. For example, if women from more deprived areas with lower vaccination uptake and lower screening coverage are more likely to have sex with people within a similar area, this will theoretically limit the potential herd protection. Therefore, the results of the serological surveillance among sexual health clinic attenders could inform consideration for mop-up vaccination in certain subgroups with apparently lower HPV vaccination coverage and/or targeting subgroups to improve cervical screening uptake.

9.3.3. Informing the decision on whether to introduce the nonavalent vaccine to the National HPV Immunisation Programme

Post-vaccination monitoring of the impact of HPV vaccination on HPV prevalence has consistently shown clear evidence of reductions in the prevalence of vaccine types. With the licencing of the nonavalent vaccine by the European Medicines Agency (EMA), the additional benefit of this vaccine against high-grade disease and cervical cancer should be considered. In the UK, we have previously shown that the high-risk types HPV16 and 18 are associated with around 82.6% of cervical cancers and the other high-risk types included in the nonavalent vaccine are associated with an additional 13.7% of cervical cancers[131]. However, an important factor to consider when comparing the likely impact of the three vaccines (bivalent, quadrivalent and nonavalent) against cervical cancer is the potential cross-protection and/or type-replacement seen with the lower valency vaccines. High cross-protection of vaccination against closely related HPV types would mean that the relative additional benefit of the nonavalent vaccine would diminish. Conversely, if there was evidence of an increase in non-vaccine types due to type replacement, then the potential impact of the nonavalent vaccine would increase and therefore its introduction would be more cost-effective. The Papillomavirus Rapid Interface for Modelling and Economics (PRIME) tool is a WHO resource developed to give users estimates of impact and cost-effectiveness of HPV vaccination. This tool has demonstrated that the effects of cross-protection and herd protection could substantially affect the cost-effectiveness estimates for introduction of the nonavalent vaccine[175]. Others have suggested that if there is a moderate cross-protective effect on non-HPV16/18 infections (such as HPV31, 33 and 45), that it may be easier to further reduce the prevalence of these HPV types in the population via herd protection, due to their lower prevalence and lower basic reproduction number[138]. The evidence in Section 6.3 of this thesis that there were substantial declines in HPV31, 33 and 45 within the post-vaccination period support this

modelling work. Whereas the bivalent vaccine will not offer the same protection against high-risk infection as the nonavalent vaccine, substantial cross-protection will certainly make the bivalent vaccine a serious competitor when countries decide which vaccine is more cost-effective for their national programme.

9.3.4. Informing the decision on whether to introduce vaccination of males

Gender neutral vaccination: In this thesis, I have not directly investigated the herd protection effect among men. I discuss this omission below in Section 9.5. However, this thesis has demonstrated a herd protection affect among unvaccinated females and, in most recent years, very low prevalence of HPV16, 18, 31, 33 and 45 (Section 6.3). These declines were even greater than vaccine coverage. These results strongly suggest that herd protection from a female vaccination programme has already reduced HPV infection in heterosexual men. These results will inform the potential additional benefit of male vaccination for the incidence of cervical cancer but also for male HPV-related cancers.

Targeted vaccination of men who have sex with men (MSM): Although there is evidence to suggest that there is some sexual mixing between some MSM and women [176, 177], the potential herd protection effect of female vaccination among MSM will be lower than the herd protection effect for heterosexual men. The results of declining HPV16 and 18 prevalence in women from this surveillance (Section 6.2) were incorporated into the cost effectiveness model for MSM vaccination which was conducted by PHE[178]. This model concluded that the quadrivalent vaccine was likely to be effective and cost-effective at reducing HPV related disease in MSM. The results of this model formed part of the evidence which led to the JCVI advising that targeted vaccination of MSM aged up to 45 years old attending sexual health and HIV clinics should be undertaken if it can be delivered at a cost-effective price[179]. As a result of this, a pilot of HPV vaccination was introduced in 2016 in 42 sexual health and HIV clinics in England. Following the results of this pilot, it was

confirmed that a nationwide HPV vaccination programme for MSM will be introduced in a phased roll-out from 2018.

9.3.5. Informing introduction of vaccination in low and middle countries

It would be remiss to present data on the impact of HPV vaccination without touching upon the burden of HPV-related disease in low and middle income countries. Whereas cervical cancer is the 13th most common cancer among females in the UK[37], worldwide it is the 4th most common and in many low and middle income countries it is the most common cancer in females[36]. The variations in the incidence of cervical cancer are largely dependent on sexual behaviours and attitudes in different countries as well as the provision of secondary prevention (i.e. availability of cervical cancer screening). Despite the disproportionately higher burden of cervical cancer in low and middle income countries, the vast majority of national HPV vaccination programmes have been established in high-income countries which will only increase this disparity. It has been estimated that up to 2014, HPV vaccination programmes had only targeted 12% of young adolescent females worldwide, 70% of which were in high-income countries[77]. Clearly those at greatest risk of cervical cancer remain the most in need of HPV vaccination.

There are mechanisms in place to assist with funding of HPV vaccination in lower income countries. Firstly, the GAVI Alliance funds vaccines for the poorest countries based on their gross national income per capita. There were 54 GAVI eligible countries in 2016[180], and by 2016, HPV vaccination had been implemented, or a demonstration programme completed, in 23 of these countries. Another mechanism is from the Pan American Health Organization (PAHO) who collectively procures vaccines for resource-poor countries in the region in order to obtain a much lower price than could be obtained if each country procured for smaller amounts of the vaccine separately.

In the end, the decision of individual countries on whether to vaccinate will depend not only on the burden of disease and the cost of procuring and delivering the vaccine but also on the safety and potential effectiveness of the vaccines. The increasing amount of data on population effectiveness following HPV vaccination in other countries can help inform the cost-effectiveness and vaccine strategies in these countries. In countries with lower resources, we perhaps need to be more inventive; for example, considering the relative benefit of the nonavalent vaccine considering the excellent cross-protection from the bivalent vaccine demonstrated in this thesis, or considering using only a single dose of vaccine. Whilst this thesis explores the impact of HPV vaccination in a high income country with high vaccination coverage, there are important lessons that can be learnt. I give two examples of prior limitations of the surveillance in England which have been addressed in this thesis and which could be of interest to other countries. Firstly, in England there are limited data on HPV vaccination status given the lack of a national registry. I have demonstrated methods to investigate changes in HPV prevalence over time and the association with estimated national coverage in the absence of a direct measure of vaccine effectiveness. Secondly, recent analyses have focussed on changes in HPV prevalence within the post-vaccination period to overcome changes in HPV testing between the pre- and post-vaccination period. Similar approaches could be taken in low and middle income countries who vaccinate without baseline data on HPV prevalence in target populations.

9.4. Implications for secondary prevention by cervical screening of vaccinated populations

The decision to screen is often considered against criteria described by Wilson and Junger in 1968[181]. The first of these criteria is that “the condition sought should be an important health problem”. The results of this thesis have clearly shown that national vaccination has reduced HPV infection and will likely have an impact on the

incidence of cervical cancer in the future. However, at least in the shorter term, cervical cancer will remain an important public health problem in the UK. Nevertheless, these substantial declines in HPV16 and 18 and some closely related HPV types will have implications for the Cervical Screening Programme in England as current screening practices are based on infection and disease rates from before the introduction of vaccination.

Another of Wilson and Junger's criteria is that "there should be a suitable test or examination". With decreases in HPV infections there will inevitably be a decrease in the positive predictive value (PPV) of both HPV testing and cervical cytology. Data from women attending for cervical screening in Scotland has shown that the PPV of cytology for CIN2+ is reduced by 16% in vaccinated women compared to unvaccinated women [182]. In 2016 it was announced that HPV primary screening would be implemented into the national cervical screening programme in England. The evidence is clear that HPV testing will have a higher PPV for CIN2+ in vaccinated populations compared to cytology[183]. However, non-vaccine HPV types have been shown to have a lower risk of subsequent disease compared to HPV16. One approach may be to triage HPV positive women with type-specific HPV testing and only refer based on HPV type-specific PPV for disease

Finally, I consider how the results of this thesis could potentially influence whether different screening intervals are offered for vaccinated and unvaccinated women. A recent simulation study concluded that fewer lifetimes screens are needed for women vaccinated with the bivalent vaccine and fewer still for those vaccinated with the nonavalent vaccine. Any future studies considering screening in the post-vaccination era should include data from population-based studies such as the one included in this thesis to allow incorporation of the protection against vaccine types, cross-protection and herd protection of the vaccine. The data from this thesis could also inform whether herd protection is sufficient to consider amending screening

strategies for all women in a highly vaccinated population regardless of their vaccination status. This could be of particular interest in England where vaccination status is not necessarily recorded accurately on the NHS call-recall system for screening (see Section 5.1.2).

9.5. Research gaps and implications for future research

This thesis focusses on the impact of the HPV vaccination programme on HPV infection and early disease outcomes. In England, the first vaccinated women entered the cervical screening programme in 2015. This offers further opportunities to monitor the impact of HPV vaccination in this population. Future surveillance will monitor changes in HPV infection in the population attending for cervical screening, who are likely to be a lower risk population than those attending for chlamydia screening. Furthermore, the surveillance programme will monitor changes in the incidence of cervical pre-cancer and cancer in the post-vaccination era. We are already conducting HPV testing of cervical cancers diagnosed under the age of 30 years old in women eligible for vaccination in order to detect changes in the relative proportion that are positive for HPV vaccine types and to remain vigilant for potential vaccine failures. Looking to the future, similar testing will be considered in non-cervical cancers which, generally, occur at older ages than cervical cancer.

Another consideration which, unfortunately, was outside of the timeframe of this PhD is the extent of protection of the vaccines against HPV infection over a longer time period. Long-term protection is expected against the vaccine types but this is less clear for the cross-protection against other HPV types. However, the results of this thesis showing such substantial declines in HPV31, 33 and 45 due to direct and indirect protection of HPV vaccination may mean that the prevalence of infection with these types becomes so low in the population that the duration of direct protection is less important.

I previously mentioned that this thesis has only considered HPV infection in females rather than males. The surveillance studies in this thesis make use of a convenient source of residual material which can be tested for HPV DNA infection. No equivalent source exists for males as chlamydia screening is performed using urine specimens for the majority of men. Urine has been shown to lack sensitivity to detect HPV infection, particularly in men[116]. Therefore, in this thesis, I have taken the approach to infer herd protection in heterosexual males based on the herd protection in females.

Finally, this thesis has only explored the impact of the bivalent HPV vaccine. With the change in the national programme to the quadrivalent vaccine in 2012, there will be similar questions on the protection of this vaccine against infection. There will also be new questions such as the direct impact of the vaccine against genital warts. This is already being seen in younger women who would have been eligible to receive the quadrivalent vaccine. In 2016 compared to 2009, there was a 72% decline in the rate of genital warts diagnoses recorded in GUMCAD among 15 to 17 year old females, the majority of whom would have been offered the quadrivalent vaccine[118]. The impact of the quadrivalent vaccine on infection and disease will be complicated by mixing between populations vaccinated with the bivalent vaccine and future analyses will need to take this into account.

9.6. Overall conclusions

National surveillance of residual specimens has offered a convenient and effective approach to provide the first results about the impact of the HPV vaccination programme in England. Surveillance using routinely collected samples and data has also some limitations which I have discussed above. In this thesis, I have described work and analyses to address some of these limitations including collection and validation of HPV vaccination records for individual women and the assessment of

changes in non-vaccine types following an assay change between the pre- and post-vaccination periods.

The high overall vaccination coverage in England has been confirmed with serological surveillance although with some variations in uptake among some subgroups of the population attending a sexual health clinic. The importance of this high coverage should not be underestimated as this affects both direct and indirect protection. This is also particularly important for other countries where vaccination coverage has been dramatically affected by safety concerns raised by anti-vaccination campaigns.

There has been some controversy around the potential protective effect of the bivalent vaccine against genital warts. This thesis contradicts ecological analyses with results from a case-control study showing no evidence of any effect of this vaccine against warts.

Results in this thesis have also shown dramatic declines in vaccine types with consistent evidence that HPV16 and 18 has declined within the post-vaccination period and, as expected, is lower in vaccinated women compared to unvaccinated women. Furthermore, a relatively unexpected result from this thesis is the substantial decline in HPV31, 33 and 45 eight years following the introduction of vaccination. This exciting finding supports the theory that non-vaccine HPV types are easier to control via herd protection and suggests that direct protection against these types may not be necessary. These five types (HPV16, 18, 31, 33 and 45) are associated with over 90% of cervical cancers in the UK[131]. Reassuringly, this thesis provides no evidence that other non-vaccine types are becoming more common following the reductions in HPV16 and 18. The encouraging results in this thesis have added to other international data on the benefits of HPV vaccination and the expectation that national HPV vaccination programmes will reduce the incidence of cervical cancer.

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Appendices

Appendix A: Systematic review and meta-analysis for changes in non-vaccine HPV types

Table A1: Inclusion and exclusion criteria for systematic review

Criteria	Inclusion	Exclusion
Study design	- Repeat cross-sectional studies/surveillance with at least one assessment of HPV infection pre-vaccine introduction and at least one assessment post-vaccine introduction	- Individually randomised trials - Cohort studies comparing HPV infection in the same women pre- and post-vaccination - Post-vaccination studies comparing HPV infection in vaccinated and unvaccinated women
Study population	- Human participants (females and/or males) - Population and recruitment were the same for pre- and post-vaccination periods	- Populations with very low vaccination coverage (<2%) in the post-vaccination period - Only considering infection in populations with HPV-related disease (e.g. cervical cancer)
HPV infection outcome	- Considering HPV DNA infection (either prevalence, odds or incidence) in relevant specimens from population	- Only HPV vaccine types were considered - Non-vaccine types were pooled and type-specific results were not available from study authors[1]

1: Authors were not contacted for the updated systematic review conducted in 4th December 2017 as results were not published. Therefore, publications were included if they considered changes of non-vaccine types regardless of whether type-specific results were not presented

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Population-Level Effects of Human Papillomavirus Vaccination Programs on Infections with Nonvaccine Genotypes

Technical Appendix

Search Details, Study Details, and Prevalence Ratios

Database Search Strategies

Medline Search Strategy: identified 2,410 studies (2016 Feb 19)

1. Epidemiologic Studies/
2. exp case-control Studies/
3. (case* and control*).tw
4. exp Cohort Studies/
5. cohort*.tw
6. Cross-sectional Studies/
7. (cross* and section*).tw
8. Seroepidemiologic Studies/
9. Sentinel Surveillance/
10. Public Health Surveillance/
11. Incidence/
12. Prevalence/
13. Odds Ratio/
14. odds ratio.tw
15. risk ratio.tw
16. rate ratio.tw
17. relative risk.tw

18. screening method.tw

19. effectiveness.tw

20. observational.tw

21. (step* and wedge*).tw

22. Or/1-21

23. Human Papillomavirus DNA Tests/

24. exp Papillomavirus Infections/

25. exp Papillomaviridae/

26. (HPV or papilloma*).tw

27. Uterine Cervical Neoplasms/

28. Genital Neoplasms, Female/

29. Genital Diseases, Female/

30. Uterine Cervical Dysplasia/

31. (Penile ADJ1 wart).tw

32. (cervi* or genit*).tw

33. warts.tw

34. condyloma*.tw

35. neoplas*.tw

36. dysplas*.tw

37. lesion*.tw

38. cancer*.tw

39. carcin*.tw

40. maligna*.tw

41. disease*.tw

42. (carcinoma adj2 situ).tw

43. Or/33-42

44. And/32,43

45. Or/23-30,44

46. (Immunis* or immuniz* or vaccin*).tw

47. Papillomavirus Vaccines/

48. Or/46-47

49. Humans/

50. limit to yr=2007-2016

51. And/22,45,48,49,50

Embase search strategy: identified 3,843 studies (2016 Feb 19)

1. Epidemiology/
2. Cross-sectional study/
3. (cross$ ADJ1 section$).tw
4. exp case control study /
5. (case$ ADJ1 control$).tw
6. cohort analysis/
7. cohort$.tw
8. exp Disease surveillance/
9. exp health survey/
10. incidence/
11. exp prevalence/
12. sentinel surveillance/
13. seroepidemiology/
14. risk/
15. infection risk/
16. population risk/
17. risk reduction/
18. observational study/
19. (odd$ ADJ1 ratio).tw
20. (risk ADJ1 ratio).tw
21. (rate ADJ1 ratio).tw

22. (relative ADJ1 risk).tw

23. (screening ADJ1 method).tw

24. effectiveness.tw

25. observational.tw

26. (step$ ADJ1 wedge$).tw

27. Or/1-26

28. exp Papilloma virus /

29. hpv.tw

30. Papilloma$.tw

31. Uterine cervix disease/

32. Uterine cervix dysplasia/

33. exp Uterine Cervix Tumor/

34. urogenital tract tumor/

35. genital tract tumor/

36. female genital tract tumor/

37. female genital tract cancer/

38. gynecologic cancer/

39. genital tract cancer/

40. female genital tract cancer/

41. Urogenital tract cancer/

42. Female genital tract cancer/

43. female genital tumor/

44. female genital tract infection/

45. genital tract infection/

46. gynecologic infection/

47. (peni$ ADJ1 wart$).tw

48. (cervi$ or genit$).tw

49. wart$.tw

50. condyloma$.tw

51. neoplas$.tw

52. dysplas$.tw

53. lesion$.tw

54. cancer$.tw

55. carcin$.tw

56. maligna$.tw

57. disease$.tw

58. (carcinoma ADJ2 situ).tw

59. Or/49-58

60. And/48,59

61. Or/28-47,60

62. (Immunis$ or immuniz$ or vaccin$).tw

63. Wart virus vaccine/

64. Or/62,63

65. Humans/

66. limit to yr=2007-2016

67. And/27,61,64,65,66

LILACS search strategy: identified 58 studies (2016 Feb 19)

((cross$ AND section$) OR (case$ AND control$) OR (cohort$) OR (odd$ AND ratio) OR (risk AND ratio) OR (rate AND ratio) OR (relative AND risk) OR effectiveness OR observational OR ("step wedge" OR "step-wedge" OR stepwedge)) AND (hpv OR Papilloma$ OR ((cervi$ or genit$) AND (wart$ OR neoplas$ OR dysplas$ OR lesion$ OR cancer$ OR carcin$ OR adeno$ OR squamous$ OR disease$ OR (carcinoma AND situ)))) AND (Immuni$ or vaccin$) AND (PD 2007 OR PD 2008 OR PD 2009 OR PD 2010 OR PD 2011 OR PD 2012 OR PD 2013 OR PD 2014 OR PD 2015 OR PD 2016)

AIM search strategy: identified 17 studies (2016 Feb 19)

hpv OR Papilloma$

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Technical Appendix Table 1. Characteristics of studies selected for systematic review and meta-analysis of changes in prevalence of nonvaccine HPV genotypes*

Characteristic	Study (reference no.)								
	Cameron et al. (*1*)	Chow et al. (*2*)	Cummings et al. (*3*)	Kahn et al. (*4*)	Markowitz et al. (*5*)	Mesher et al. (*6*)	Söderlund-Strand et al. (*7*)	Sonnenberg et al. (*8*)	Tabrizi et al. (*9*)
Country of study	Scotland UK	Australia	USA	USA	USA	England, UK	Sweden	Great Britain, UK	Australia
Vaccine introduced	Bivalent	Quadrivalent	Quadrivalent	Quadrivalent	Quadrivalent	Bivalent	Quadrivalent	Bivalent	Quadrivalent
Sample collection, y									
Prevac	2009–2010	2004–2007	1995–2005	2006–2007	2003–2006	2008	2008	1999–2001	2005–2007
Postvac	2011–2013	2007–2014	2010	2009–2010	2009–2012	2010–2013	2012–2013	2010–2012	2010–2011
Specimens tested, no.									
Prevac	2,705	136	150	365	1,795	2,354	11,457	328	202
Postvac	3,010	328	75	383	1,209	7,321	3,555	795	1,058
Study population † age, y (additional detail)	20–21	≤ 21 (Australian born)	14–17	13–26 (had had sexual intercourse)	14–24	16–25 (sexually active)	All ages	18–44 (sexually experienced)	18–24
Setting for recruiting participants	Cervical screening as part of national cervical screening program	Chlamydia screening at sexual health center in Melbourne, Australia (tested positive)	1 of 3 primary care clinics in Indiana	Hospital-based adolescent clinic and a community health center	Population-based NHANES survey	Chlamydia screening at community sexual health settings	Chlamydia screening in a defined region of Sweden	Households participating in Natsal survey (selected with a stratified probability sample survey)	Cervical screening at sentinel family planning clinics in Sydney, Melbourne, and Perth
Specimen type	Residual LBC	Cervical and high vaginal swab	Self-collected vaginal swab	Cervicovaginal swabs by clinician or self-collected swab	Self-collected cervicovaginal swab	Residual vulval vaginal swab	Genital swabs (alone or immersed in urine)	Urine	Exfoliated cervical cells preserved in PreservCyt‡
Assay for HPV DNA testing	Multimetrix HPV assay	PapType HPV assay	Linear Array HPV Genotyping test	Linear Array HPV Genotyping test	Linear Array HPV Genotyping test	Prevac: Linear Array HPV Genotyping test in those testing positive for Hybrid Capture 2 Postvac: In-house multiplex PCR- and Luminex-based genotyping system	PCR testing with genotyping by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry	In-house multiplex PCR- and Luminex-based genotyping system	Amplicor DNA test for 13 high-risk types (if negative, tested for presence of mucosal DNA by using L1 consensus primer set PGMY09-PGMY11). If positive for Amplicor or PCMY09/PGMY11, PCR-ELISA were genotyped by using the Linear Array HPV genotyping test
Demographic and sexual behavior data collected	Scottish Index of Multiple Deprivation, mo/y of birth§	Age-stratified PRs were adjusted for by no.male partners, 100%	Samples matched on basis of age at enrollment, clinic site,	Age, race, health care insurance, knowledge of HPV vaccines,	Ethnicity, poverty index, and for those reporting ever having sex, age at first sex,	Age- stratified PRs were adjusted for age, chlamydia positivity at	All samples were anonymised (individual age was known)	Extensive demographic and sexual behavior data collected‡	Age, current use of hormonal contraception, smoking status and postal code

Characteristic	Study (reference no.)								
	Cameron et al. (*1*)	Chow et al. (*2*)	Cummings et al. (*3*)	Kahn et al. (*4*)	Markowitz et al. (*5*)	Mesher et al. (*6*)	Söderlund-Strand et al. (*7*)	Sonnenberg et al. (*8*)	Tabrizi et al. (*9*)
		condom use with all partners in the past 12 mo status, and anatomic sampling method (cervical vs. high vaginal sample)	and reported sexual activity. Data on ethnicity, no. sexual partners in last year and in last 2 mo, no. lifetime sexual partners, no. instances of vaginal intercourse in last year and in the last 2 mo§	smoking status, gynecologic history (no. pregnancies, history of STIs), sexual behaviors (i.e., age at first sex, no. male lifetime partners, no. male partners in last 3 mo, anal sex, condom use)§	lifetime no. partners, no. partners in last 12 mo§	time specimen taken, and collection venue type			of residence§
Vaccination information	Linked from Scottish Immunisation call/recall system and Child Health Schools Programme system	Self-reported; not available for all women	Collected from medical notes	Collected from immunisation registry for 87% of women; collected from self-administered questionnaire for others	Self-reported	Not collected for individuals	Not collected for individuals	Self-reported	Self-reported and validated against the National HPV vaccine register

*Study design for all studies was repeat cross-sectional. HPV, human papillomavirus; LBC, liquic-based cytology; Prevac, prevaccination period; Postvac, postvaccination period; PRs, prevalence ratios.
†Population in all studies were female.
‡Cytyc Corporation, Marlborough, MA, USA.
§These data were not used to adjust the HPV prevalence ratios in this meta-analysis.

Technical Appendix Table 2. Prevalence ratios for nonvaccine high-risk HPV types for female adolescents and women in systemic review and meta-analysis, by age group and vaccine type*

	Bivalent vaccine				Quadrivalent vaccine			
		Heterogeneity				Heterogeneity		
Age group, y/HPV type	No. of studies†	I^2, %	p value	Prevalence ratio (95% CI)	No. studies†	I^2, %	p value	Prevalence ratio (95% CI)
≤19								
Nonavlent vaccine HPV types	2				6			
HPV 31		10.4	0.291	0.54 (0.29–1.03)		8.7	0.36	0.75 (0.60–0.96)
HPV 33		0	0.785	1.66 (0.94–2.92)		0	0.687	0.89 (0.64–1.24)
HPV 45		75.4	0.044	–		0	0.716	1.01 (0.76–1.34)
HPV 52		0	0.408	1.93 (1.34–2.77)		0	0.627	1.20 (0.99–1.47)
HPV 58		0	0.445	1.19 (0.81–1.73)		0	0.742	0.92 (0.69–1.22)
Other high-risk HPV types	2				6			
HPV 35		85.2	0.009	–		0	0.914	0.91 (0.58–1.42)
HPV 39		0	0.755	1.30 (0.89–1.91)		0	0.932	1.26 (1.01–1.58)
HPV 51		74.9	0.046	–		35.2	0.172	1.16 (1.00–1.36)
HPV 56		18.3	0.269	2.08 (1.43–3.04)		64.9	0.014	–
HPV 59		51.9	0.149	–		0	0.478	1.27 (1.03–1.57)
HPV 68		0	0.444	1.84 (0.62–5.47)		0	0.601	1.20 (0.82–1.76)
Other possibly high-risk types	2				4			
HPV 26		0	0.873	1.89 (0.84–4.26)		26.8	0.251	1.21 (0.38–3.81)
HPV 53		0	0.894	2.22 (1.25–3.94)		0	0.445	1.28 (0.88–1.85)
HPV 70		0	0.957	4.07 (1.43–11.55)		0	0.97	0.82 (0.41–1.64)
HPV 73		0	0.926	1.39 (0.98–1.98)		0	0.806	1.32 (0.83–2.07)
HPV 82		0	0.998	2.00 (0.50–7.95)		65.1	0.035	–
20–24								
Nonavalent vaccine HPV types	3				5			
HPV 31		57.8	0.094	–		0	0.889	0.95 (0.81–1.10)
HPV 33		55.0	0.108	–		48.1	0.103	–
HPV 45		74.2	0.021	–		56.9	0.055	–
HPV 52		65.6	0.055	1.26 (0.87–1.83)		0	0.53	1.28 (1.12–1.46)
HPV 58		0	0.499	1.17 (0.94–1.46)		0	0.684	1.12 (0.93–1.34)
Other high-risk HPV types	3				5			
HPV 35		0	0.968	1.22 (0.79–1.87)		43.1	0.134	–
HPV 39		44.8	0.163	1.32 (0.93, 1.88)		0	0.743	1.09 (0.93–1.28)
HPV 51		0	0.57	1.37 (1.16–1.62)		47.0	0.11	1.19 (0.88–1.61)
HPV 56		75.4	0.017	1.45 (0.82–2.59)		87.5	<0.001	–
HPV 59		86.1	0.001	–		0	0.604	1.13 (0.94–1.37)
HPV 68		67.4	0.046	–		0	0.842	0.99 (0.72–1.37)
Other possibly high-risk types	3				3			
HPV 26		69.0	0.04	–		21.1	0.282	1.35 (0.28–6.47)
HPV 53		0.3	0.367	1.23 (1.05–1.45)		16.9	0.3	0.90 (0.64–1.25)
HPV 70		0	0.382	1.11 (0.81–1.51)		0	0.811	2.47 (1.24–4.94)
HPV 73		43.8	0.169	–		76.3	0.015	–
HPV 82		73.7	0.022	–		0	0.989	0.94 (0.39–2.26)

*HPV, human papillomavirus; –, prevalence ratios were not calculated because of heterogeneity of data.
†Number of studies were the same for all HPV types within each category.

Technical Appendix Table 3. Prevalence ratios for nonvaccine high-risk HPV types for female adolescents and women in systemic review and meta-analysis, by age group and potential bias*

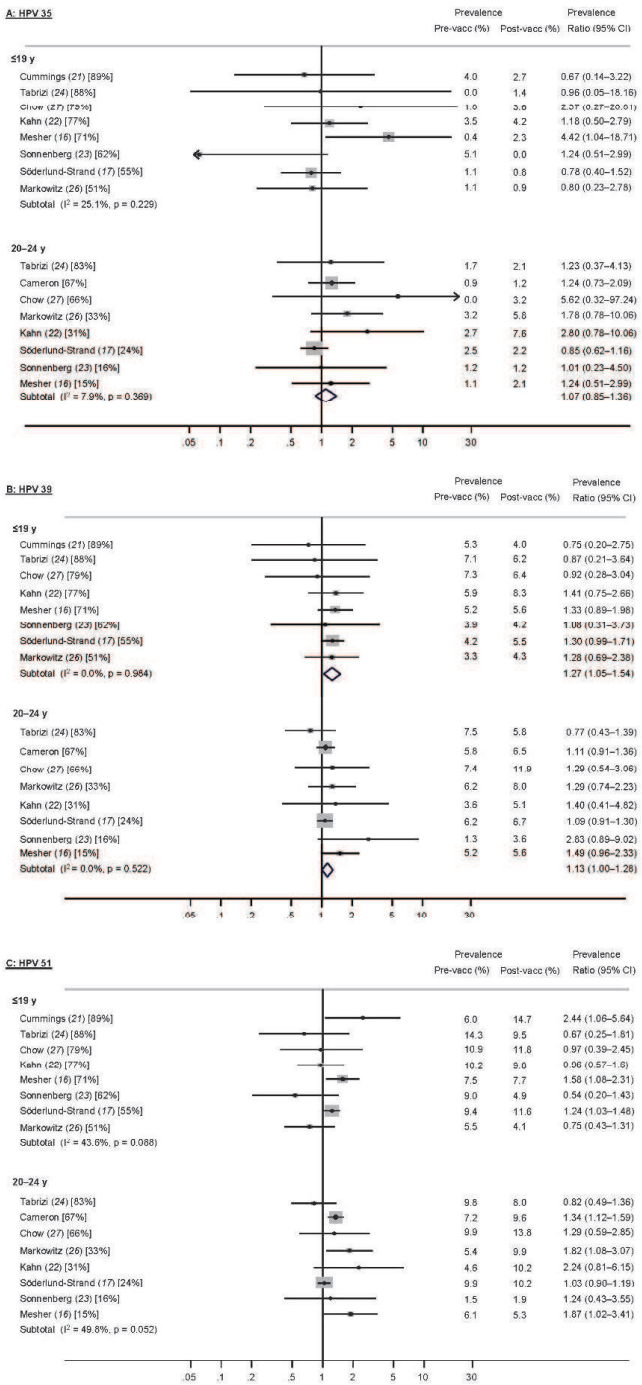
	Relatively low potential bias†				Relatively high potential bias‡			
		Heterogeneity				Heterogeneity		
Age group, y/HPV type	No. studies§	I^2,%	p value	Prevalence ratio (95% CI)	No. studies§	I^2,%	p value	Prevalence ratio (95% CI)
≤19								
Nonavalent vaccine HPV types	5				3			
HPV 31		31.2	0.213	–		0	0.447	0.73 (0.58–0.93)
HPV 33		0	0.526	0.79 (0.30–2.06)		34.4	0.218	–
HPV 45		21.5	0.278	0.84 (0.49–1.44)		0.6	0.366	0.99 (0.76–1.31)
HPV 52		0	0.681	1.09 (0.77–1.56)		61.9	0.072	–
HPV 58		0	0.672	0.87 (0.58–1.30)		0	0.505	1.08 (0.82–1.42)
Other high-risk HPV types	5				3			
HPV 35		0	0.424	0.85 (0.46–1.58)		60.6	0.079	–
HPV 39		0	0.907	1.21 (0.83–1.78)		0	0.846	1.30 (1.04–1.61)
HPV 51		45.3	0.120	–		0	0.433	1.28 (1.09–1.50)
HPV 56		69.3	0.011	–		79.9	0.007	–
HPV 59		0	0.465	1.29 (0.94–1.76)		85.9	0.001	–
HPV 68		12.6	0.333	1.21 (0.76–1.93)		0	0.948	1.33 (0.75–2.36)
Other possibly high-risk types	5				1			
HPV 26		3.3	0.388	1.27 (0.45–3.58)		–	–	1.93 (0.82–4.59)
HPV 53		0	0.514	1.32 (0.92–1.90)		–	–	2.19 (1.18–4.04)
HPV 70		0	0.831	0.90 (0.45–1.76)		–	–	4.02 (1.31–12.32)
HPV 73		0	0.909	1.33 (0.87–2.05)		–	–	1.39 (0.96–2.00)
HPV 82		55.0	0.064	–		–	–	2.00 (0.42–9.44)
20–24								
Nonavalent vaccine HPV types	5				3			
HPV 31		27.7	0.237	–		0	0.670	0.95 (0.81–1.11)
HPV 33		0	0.599	0.64 (0.52–0.78)		0	0.424	1.03 (0.83–1.27)
HPV 45		78.5	0.001	–		0	0.948	0.90 (0.74–1.10)
HPV 52		0	0.905	1.06 (0.91–1.22)		11.8	0.322	1.37 (1.20–1.56)
HPV 58		0	0.859	1.04 (0.85–1.28)		0	0.600	1.23 (1.02–1.50)
Other high-risk HPV types	5				3			
HPV 35		0	0.754	1.42 (0.97–2.08)		10.7	0.326	0.90 (0.67–1.21)
HPV 39		8.3	0.359	1.12 (0.94–1.34)		0	0.415	1.14 (0.97–1.34)
HPV 51		32.5	0.205	–		46.9	0.152	–
HPV 56		0	0.914	1.03 (0.89–1.21)		94.5	0.000	–
HPV 59		0	0.443	1.08 (0.91–1.28)		86.4	0.001	–
HPV 68		0	0.692	1.04 (0.72–1.49)		72.5	0.026	–
Other possibly high-risk types	5				1			
HPV 26		54.8	0.065	–		–	–	1.14 (0.37–3.50)
HPV 53		36.3	0.179	–		–	–	1.52 (0.86–2.69)
HPV 70		34.5	0.191	–		–	–	1.64 (0.79–3.37)
HPV 73		56.0	0.059	–		–	–	1.92 (1.04–3.53)
HPV 82		0	0.984	0.75 (0.60–0.94)		–	–	0.22 (0.10–0.51)

*HPV, human papillomavirus; –, prevalence ratios were not calculated because of heterogeneity of data.
†Average-low potential bias includes 6 studies (*1*, *3*–*5*, *8*, *9*).
‡Average-high potential bias includes 3 studies (*2*,*6*,*7*).
§Number of studies were the same for all HPV types within each category.

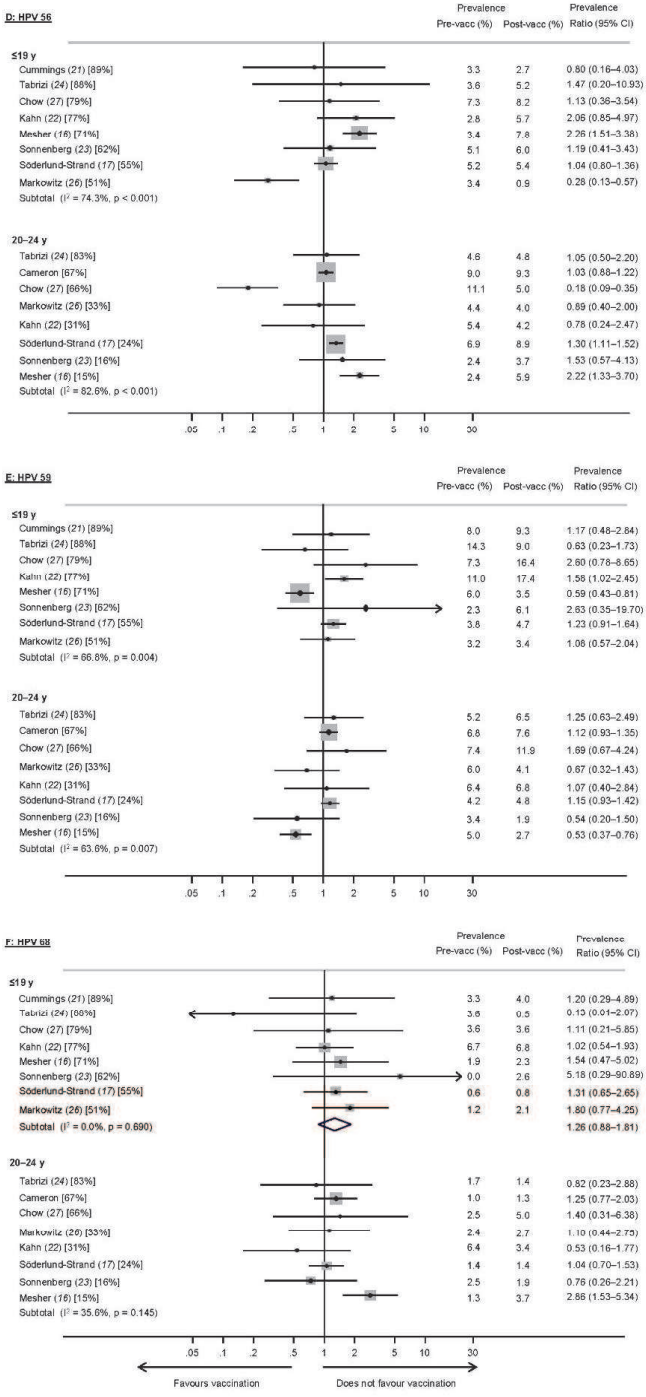
Technical Appendix Table 4. Prevalence ratio for nonvaccine high-risk HPV types for female adolescents and women in systemic review and meta-analysis, by age group and vaccination coverage*

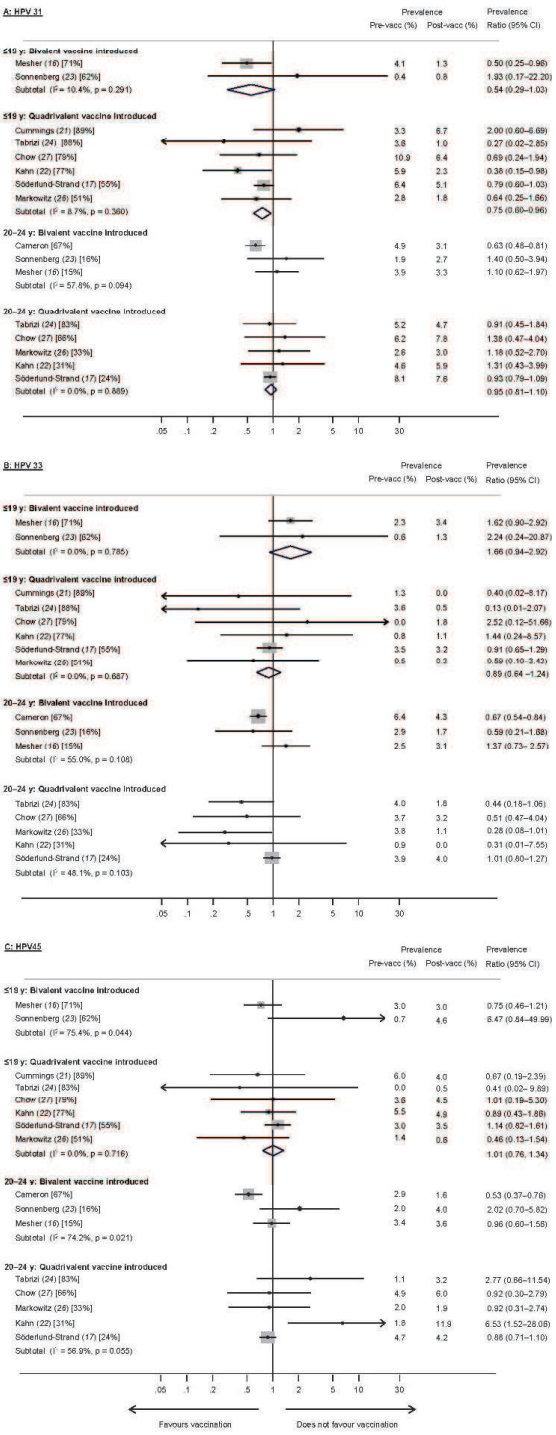
	Low vaccination coverage (<50%)				High vaccination coverage (≥50%)			
	No.	Heterogeneity		Prevalence ratio	No.	Heterogeneity		Prevalence ratio
Age group, y/ HPV type	studies†	I^2, %	p value	(95% CI)	studies†	I^2, %	p value	(95% CI)
≤19								
Nonavalent HPV types	0				8			
HPV 31		–	–	–		6.4	0.381	0.73 (0.58–0.91)
HPV 33		–	–	–		0	0.471	1.04 (0.78–1.38)
HPV 45		–	–	–		5.5	0.387	0.96 (0.75–1.23)
HPV 52		–	–	–		24.0	0.238	1.34 (1.13–1.59)
HPV 58		–	–	–		0	0.727	1.01 (0.80–1.26)
Other high-risk HPV types	0				8			
HPV 35		–	–	–		25.1	0.229	–
HPV 39		–	–	–		0	0.984	1.27 (1.05–1.54)
HPV 51		–	–	–		43.6	0.088	–
HPV 56		–	–	–		74.3	<0.001	–
HPV 59		–	–	–		66.8	0.004	–
HPV 68		–	–	–		0	0.690	1.26 (0.88–1.81)
Other possibly high-risk types	0				6			
HPV 26		–	–	–		0	0.478	1.63 (0.84–3.16)
HPV 53		–	–	–		3.6	0.394	1.51 (1.10–2.06)
HPV 70		–	–	–		23.6	0.257	1.34 (0.75–2.39)
HPV 73		–	–	–		0	0.961	1.36 (1.03–1.80)
HPV 82		–	–	–		49.0	0.081	–
20–24								
Nonavalent HPV types	5				3			
HPV 31		0	0.838	0.96 (0.83–1.12)		25.5	0.261	–
HPV 33		36.3	0.179	–		0	0.618	0.65 (0.53–0.81)
HPV 45		55.9	0.06	–		62.7	0.068	–
HPV 52		26.1	0.248	–		0	0.513	1.10 (0.94–1.27)
HPV 58		0	0.689	1.21 (1.01–1.45)		0	0.807	1.04 (0.83–1.30)
Other high-risk HPV types	5				3			
HPV 35		30.4	0.219	–		0	0.590	1.29 (0.80–2.07)
HPV 39		5.3	0.377	1.17 (1.00–1.37)		0	0.482	1.08 (0.89–1.30)
HPV 51		56.7	0.056	–		37.8	0.201	–
HPV 56		30.5	0.218	–		91.7	<0.001	–
HPV 59		73.5	0.004	–		0	0.673	1.15 (0.96–1.37)
HPV 68		61.7	0.034	–		0	0.810	1.20 (0.78–1.85)
Other possibly high-risk types	4			–	2			
HPV 26		53.8	0.09	–		0	0.862	1.76 (1.00–3.12)
HPV 53		0	0.522	1.31 (0.95–1.81)		76.6	0.039	–
HPV 70		11.8	0.334	1.72 (1.06–2.79)		0	0.335	1.08 (0.76–1.53)
HPV 73		52.5	0.097	–		0	0.503	1.02 (0.82–1.26)
HPV 82		33.7	0.21	–		0	0.675	0.75 (0.59–0.94)

*HPV, human papillomavirus; –, prevalence ratios were not calculated because of heterogeneity of data.
†Number of studies were the same for all HPV types within each category.

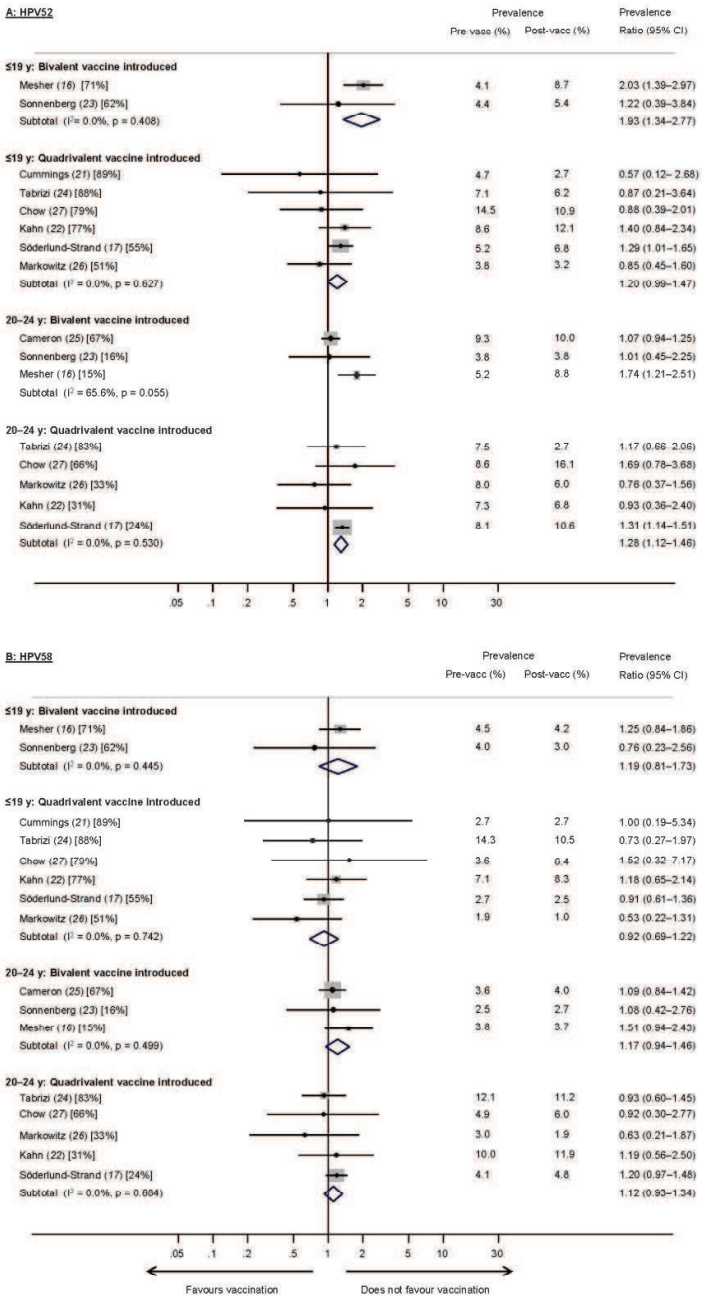


Technical Appendix Figure 1.
Prevalence ratios for meta-analysis of changes in other probable high-risk human papillomavirus (HPV) types (HPV35, HPV39, HPV51, HPV56, HPV59, and HPV68) for girls and women, by age group (≤19 and 20–24 years of age). Percentages in square brackets represent vaccination coverage (at least 1 dose) for each study and age group. The size of the dark boxes around the plot points indicates the relative weight given to each study in calculation of the summary estimate. The study by Cameron et al. (*25*) is omitted from analyses for the younger age group because this study included no data for those ≤19 years of age. The study by Cummings et al. (*21)* is omitted from analyses for women 20–24 years of age because this study included no data for this age group. Pre, prevaccination; post, postvaccination.

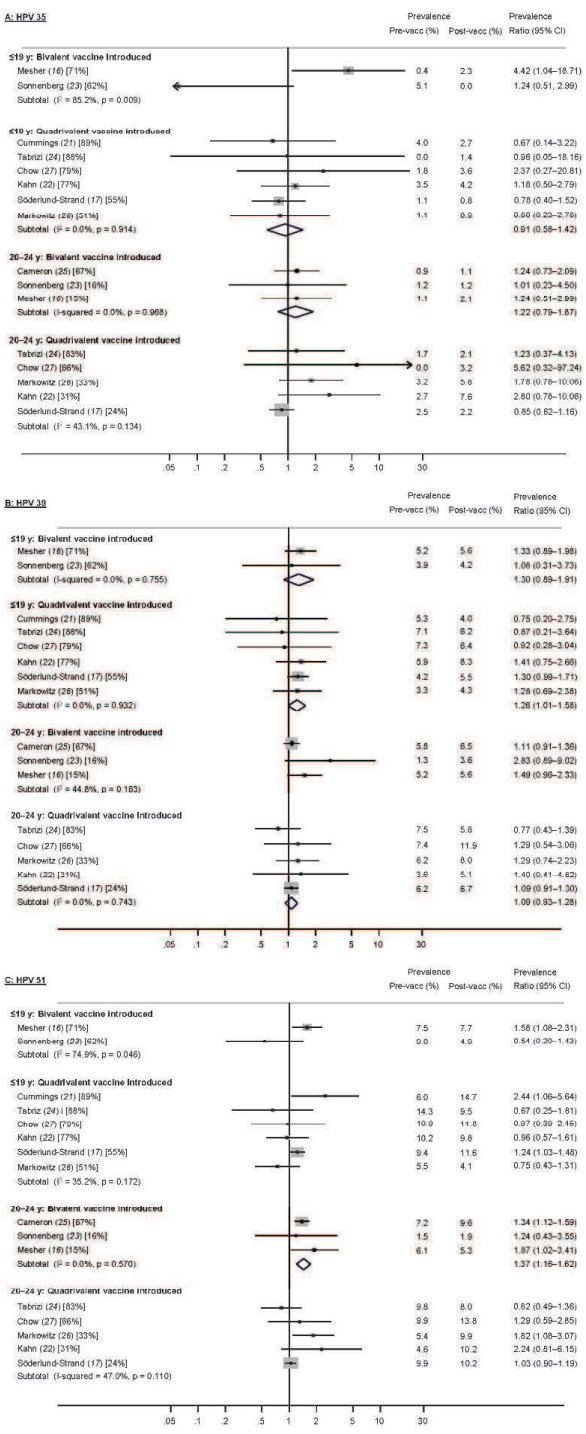

D: HPV 56
Prevalence
Pre-vacc (%)
Post-vacc (%)
Prevalence
Ratio (95% CI)
≤19 y
Cummings (21) [89%] 3.3 2.7 0.80 (0.16–4.03)
Tabrizi (24) [88%] 3.6 5.2 1.47 (0.20–10.93)
Chow (27) [79%] 7.3 8.2 1.13 (0.38–3.54)
Kahn (22) [77%] 2.8 5.7 2.06 (0.85–4.97)
Mesher (16) [71%] 3.4 7.8 2.26 (1.51–3.38)
Sonnenberg (23) [62%] 5.1 6.0 1.19 (0.41–3.43)
Söderlund-Strand (17) [55%] 5.2 5.4 1.04 (0.80–1.36)
Markowitz (26) [51%] 3.4 0.9 0.28 (0.13–0.57)
Subtotal (I² = 74.3%, p < 0.001)
20–24 y
Tabrizi (24) [83%] 4.6 4.8 1.05 (0.50–2.20)
Cameron [67%] 9.0 9.3 1.03 (0.88–1.22)
Chow (27) [66%] 11.1 5.0 0.18 (0.09–0.35)
Markowitz (26) [33%] 4.4 4.0 0.89 (0.40–2.00)
Kahn (22) [31%] 5.4 4.2 0.78 (0.24–2.47)
Söderlund-Strand (17) [24%] 6.9 8.9 1.30 (1.11–1.52)
Sonnenberg (23) [16%] 2.4 3.7 1.53 (0.57–4.13)
Mesher (16) [15%] 2.4 5.9 2.22 (1.33–3.70)
Subtotal (I² = 82.6%, p < 0.001)
.05 .1 .2 .5 1 2 5 10 30
E: HPV 59
Prevalence
Pre-vacc (%)
Post-vacc (%)
Prevalence
Ratio (95% CI)
≤19 y
Cummings (21) [89%] 8.0 9.3 1.17 (0.48–2.84)
Tabrizi (24) [88%] 14.3 9.0 0.63 (0.23–1.73)
Chow (27) [79%] 7.3 16.4 2.60 (0.78–8.65)
Kahn (22) [77%] 11.0 17.4 1.58 (1.02–2.45)
Mesher (16) [71%] 6.0 3.5 0.59 (0.43–0.81)
Sonnenberg (23) [62%] 2.3 6.1 2.63 (0.35–19.70)
Söderlund-Strand (17) [55%] 3.8 4.7 1.23 (0.91–1.64)
Markowitz (26) [51%] 3.2 3.4 1.06 (0.57–2.04)
Subtotal (I² = 66.8%, p = 0.004)
20–24 y
Tabrizi (24) [83%] 5.2 6.5 1.25 (0.63–2.49)
Cameron [67%] 6.8 7.6 1.12 (0.93–1.35)
Chow (27) [66%] 7.4 11.9 1.69 (0.67–4.24)
Markowitz (26) [33%] 6.0 4.1 0.67 (0.32–1.43)
Kahn (22) [31%] 6.4 6.8 1.07 (0.40–2.84)
Söderlund-Strand (17) [24%] 4.2 4.8 1.15 (0.93–1.42)
Sonnenberg (23) [16%] 3.4 1.9 0.54 (0.20–1.50)
Mesher (16) [15%] 5.0 2.7 0.53 (0.37–0.76)
Subtotal (I² = 63.6%, p = 0.007)
.05 .1 .2 .5 1 2 5 10 30
F: HPV 68
Prevalence
Pre-vacc (%)
Post-vacc (%)
Prevalence
Ratio (95% CI)
≤19 y
Cummings (21) [89%] 3.3 4.0 1.20 (0.29–4.89)
Tabrizi (24) [88%] 3.6 0.5 0.13 (0.01–2.07)
Chow (27) [79%] 3.6 3.6 1.11 (0.21–5.85)
Kahn (22) [77%] 6.7 6.8 1.02 (0.54–1.93)
Mesher (16) [71%] 1.9 2.3 1.54 (0.47–5.02)
Sonnenberg (23) [62%] 0.0 2.6 5.18 (0.29–90.89)
Söderlund-Strand (17) [55%] 0.6 0.8 1.31 (0.65–2.65)
Markowitz (26) [51%] 1.2 2.1 1.80 (0.77–4.25)
Subtotal (I² = 0.0%, p = 0.690) 1.26 (0.88–1.81)
20–24 y
Tabrizi (24) [83%] 1.7 1.4 0.82 (0.23–2.88)
Cameron [67%] 1.0 1.3 1.25 (0.77–2.03)
Chow (27) [66%] 2.5 5.0 1.40 (0.31–6.38)
Markowitz (26) [33%] 2.4 2.7 1.10 (0.44–2.75)
Kahn (22) [31%] 6.4 3.4 0.53 (0.16–1.77)
Söderlund-Strand (17) [24%] 1.4 1.4 1.04 (0.70–1.53)
Sonnenberg (23) [16%] 2.5 1.9 0.76 (0.26–2.21)
Mesher (16) [15%] 1.3 3.7 2.86 (1.53–5.34)
Subtotal (I² = 35.6%, p = 0.145)
.05 .1 .2 .5 1 2 5 10 30
Favours vaccination
Does not favour vaccination



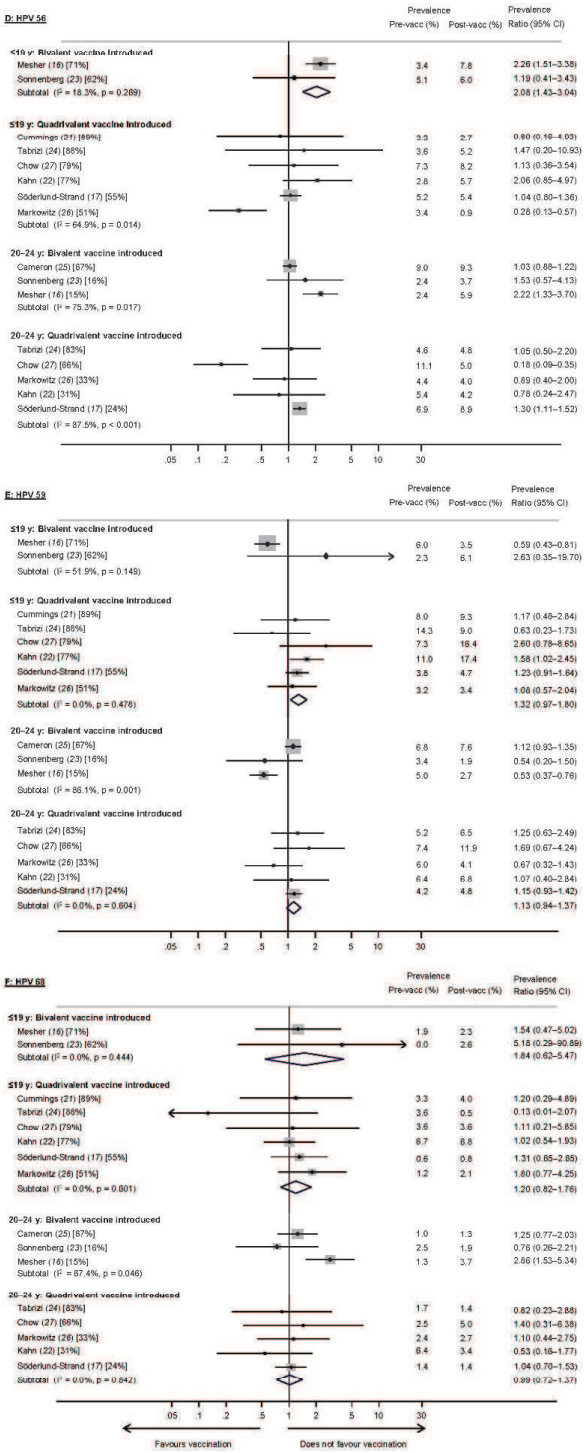
Technical Appendix Figure 2. Prevalence ratios for meta-analysis of changes in high-risk human papillomavirus (HPV) types (HPV31, HPV33, and HPV45) with evidence of cross-protection for girls and women, by age group (≤19 and 20–24 years of age) and vaccine type. Percentages in square brackets represent vaccination coverage (at least 1 dose) for each study and age group. The size of the dark boxes around the plot points indicates the relative weight given to each study in the calculation of the summary estimate. The study by Cameron et al. (*25*) is omitted from analyses for the younger age group because this study included no data for the group ≤19 years of age. The study by Cummings et al. (*21)* is omitted from analyses for women 20–24 years of age because this study included no data for this age group. Pre, prevaccination; post, postvaccination.



Technical Appendix Figure 3. Prevalence ratios for meta-analysis of changes in other high-risk human papillomavirus (HPV) types(HPV52 and HPV58) included in the nonavalent vaccine for girls and women, by age group (≤19 and 20–24 years of age) and vaccine type. Percentages in square brackets represent vaccination coverage (at least 1 dose) for each study and age group. The size of the dark boxes around the plot points indicates the relative weight given to each study in the calculation of the summary estimate. The study by Cameron et al. (*25*) is omitted from analyses for the younger age group because this study included no data for the group ≤19 years of age. The study by Cummings et al. (*21)* is omitted from analyses for women 20–24 years of age because this study included no data for this age group. Pre, prevaccination; post, postvaccination.



Technical Appendix Figure 4. Prevalence ratios for meta-analysis of changes in other probably high-risk HPV types (HPV35, HPV39, HPV51, HPV56, HPV59, and HPV68) for girls and women, by age-group (≤19 and 20–24 years of age) and vaccine type. Percentages in square brackets represent vaccination coverage (at least 1 dose) for each study and age group. The size of the dark boxes around the plot points indicates the relative weight given to each study in the calculation of the summary estimate. The study by Cameron et al. (*25*) is omitted from analyses for the younger age group because this study included no data for the group ≤19 years of age. The study by Cummings et al. (*21)* is omitted from analyses for women 20–24 years of age because this study included no data for this age group. Pre, prevaccination; post, postvaccination.


D: HPV 56
Prevalence Pre-vacc (%)
Prevalence Post-vacc (%)
Prevalence Ratio (95% CI)
≤19 y: Bivalent vaccine introduced
Mesher (16) [71%] 3.4 7.8 2.26 (1.51–3.38)
Sonnenberg (23) [62%] 5.1 6.0 1.19 (0.41–3.43)
Subtotal (I² = 18.3%, p = 0.269) 2.08 (1.43–3.04)
≤19 y: Quadrivalent vaccine introduced
Cummings (21) [89%] 3.3 2.7 0.80 (0.16–4.03)
Tabrizi (24) [88%] 3.6 5.2 1.47 (0.20–10.93)
Chow (27) [79%] 7.3 8.2 1.13 (0.36–3.54)
Kahn (22) [77%] 2.8 5.7 2.06 (0.85–4.97)
Söderlund-Strand (17) [55%] 5.2 5.4 1.04 (0.80–1.36)
Markowitz (26) [51%] 3.4 0.9 0.28 (0.13–0.57)
Subtotal (I² = 64.9%, p = 0.014)
20–24 y: Bivalent vaccine introduced
Cameron (25) [67%] 9.0 9.3 1.03 (0.88–1.22)
Sonnenberg (23) [16%] 2.4 3.7 1.53 (0.57–4.13)
Mesher (16) [15%] 2.4 5.9 2.22 (1.33–3.70)
Subtotal (I² = 75.3%, p = 0.017)
20–24 y: Quadrivalent vaccine introduced
Tabrizi (24) [83%] 4.6 4.8 1.05 (0.50–2.20)
Chow (27) [66%] 11.1 5.0 0.18 (0.09–0.35)
Markowitz (26) [33%] 4.4 4.0 0.89 (0.40–2.00)
Kahn (22) [31%] 5.4 4.2 0.78 (0.24–2.47)
Söderlund-Strand (17) [24%] 6.9 8.9 1.30 (1.11–1.52)
Subtotal (I² = 87.5%, p < 0.001)
.05 .1 .2 .5 1 2 5 10 30
E: HPV 59
Prevalence Pre-vacc (%)
Prevalence Post-vacc (%)
Prevalence Ratio (95% CI)
≤19 y: Bivalent vaccine introduced
Mesher (16) [71%] 6.0 3.5 0.59 (0.43–0.81)
Sonnenberg (23) [62%] 2.3 6.1 2.63 (0.35–19.70)
Subtotal (I² = 51.9%, p = 0.149)
≤19 y: Quadrivalent vaccine introduced
Cummings (21) [89%] 8.0 9.3 1.17 (0.48–2.84)
Tabrizi (24) [88%] 14.3 9.0 0.63 (0.23–1.73)
Chow (27) [79%] 7.3 19.4 2.60 (0.78–8.65)
Kahn (22) [77%] 11.0 17.4 1.58 (1.02–2.45)
Söderlund-Strand (17) [55%] 3.8 4.7 1.23 (0.91–1.64)
Markowitz (26) [51%] 3.2 3.4 1.08 (0.57–2.04)
Subtotal (I² = 0.0%, p = 0.478) 1.32 (0.97–1.80)
20–24 y: Bivalent vaccine introduced
Cameron (25) [67%] 6.8 7.6 1.12 (0.93–1.35)
Sonnenberg (23) [16%] 3.4 1.9 0.54 (0.20–1.50)
Mesher (16) [15%] 5.0 2.7 0.53 (0.37–0.76)
Subtotal (I² = 86.1%, p = 0.001)
20–24 y: Quadrivalent vaccine introduced
Tabrizi (24) [83%] 5.2 6.5 1.25 (0.63–2.49)
Chow (27) [66%] 7.4 11.9 1.69 (0.67–4.24)
Markowitz (26) [33%] 6.0 4.1 0.67 (0.32–1.43)
Kahn (22) [31%] 6.4 6.8 1.07 (0.40–2.84)
Söderlund-Strand (17) [24%] 4.2 4.8 1.15 (0.93–1.42)
Subtotal (I² = 0.0%, p = 0.604) 1.13 (0.94–1.37)
.05 .1 .2 .5 1 2 5 10 30
F: HPV 68
Prevalence Pre-vacc (%)
Prevalence Post-vacc (%)
Prevalence Ratio (95% CI)
≤19 y: Bivalent vaccine introduced
Mesher (16) [71%] 1.9 2.3 1.54 (0.47–5.02)
Sonnenberg (23) [62%] 0.0 2.6 5.18 (0.29–90.89)
Subtotal (I² = 0.0%, p = 0.444) 1.84 (0.62–5.47)
≤19 y: Quadrivalent vaccine introduced
Cummings (21) [89%] 3.3 4.0 1.20 (0.29–4.89)
Tabrizi (24) [88%] 3.6 0.5 0.13 (0.01–2.07)
Chow (27) [79%] 3.6 3.6 1.11 (0.21–5.85)
Kahn (22) [77%] 6.7 6.8 1.02 (0.54–1.93)
Söderlund-Strand (17) [55%] 0.6 0.8 1.31 (0.65–2.65)
Markowitz (26) [51%] 1.2 2.1 1.80 (0.77–4.25)
Subtotal (I² = 0.0%, p = 0.801) 1.20 (0.82–1.76)
20–24 y: Bivalent vaccine introduced
Cameron (25) [67%] 1.0 1.3 1.25 (0.77–2.03)
Sonnenberg (23) [16%] 2.5 1.9 0.76 (0.26–2.21)
Mesher (16) [15%] 1.3 3.7 2.86 (1.53–5.34)
Subtotal (I² = 67.4%, p = 0.046)
20–24 y: Quadrivalent vaccine introduced
Tabrizi (24) [83%] 1.7 1.4 0.82 (0.23–2.88)
Chow (27) [66%] 2.5 5.0 1.40 (0.31–6.38)
Markowitz (26) [33%] 2.4 2.7 1.10 (0.44–2.75)
Kahn (22) [31%] 6.4 3.4 0.53 (0.19–1.77)
Söderlund-Strand (17) [24%] 1.4 1.4 1.04 (0.70–1.53)
Subtotal (I² = 0.0%, p = 0.842) 0.99 (0.72–1.37)
.05 .1 .2 .5 1 2 5 10 30
Favours vaccination
Does not favour vaccination

Appendix C: Comparison of pre- and post-vaccination HPV infection

C1: Validation study comparing pre- and post-vaccination HPV assays

(for information only; this study was conducted by VRD at PHE and these results do not form part of this PhD).

	Result of HPV assays			
HPV type	Both tests positive	HC2/LA[1] only	Luminex[2] only	Both tests negative
Any HPV	239 (55.8%)	3 (0.7%)	73 (17.1%)	113 (26.4%)
High-risk HPV	185 (43.2%)	11 (2.6%)	36 (8.4%)	196 (45.8%)
High-risk HPV (not 16/18)	91 (21.3%)	10 (2.3%)	28 (6.5%)	299 (69.9%)
Vaccine HPV types				
HPV16 and/or HPV18	84 (19.6%)	11 (2.6%)	18 (4.2%)	315 (73.6%)
HPV16	62 (14.5%)	7 (1.6%)	16 (3.7%)	343 (80.1%)
HPV18	29 (6.8%)	11 (2.6%)	5 (1.2%)	383 (89.5%)
Nonavalent HPV types				
HPV31/33/45/52/58	81 (18.9%)	10 (2.3%)	18 (4.2%)	319 (74.5%)
HPV31/33/45	38 (8.9%)	11 (2.6%)	10 (2.3%)	369 (86.2%)
HPV31	16 (3.7%)	7 (1.6%)	2 (0.5%)	403 (94.2%)
HPV33	11 (2.6%)	3 (0.7%)	4 (0.9%)	410 (95.8%)
HPV45	13 (3.0%)	3 (0.7%)	7 (1.6%)	405 (94.6%)
HPV52	32 (7.5%)	3 (0.7%)	14 (3.3%)	379 (88.6%)
HPV58	15 (3.5%)	4 (0.9%)	0 (0%)	409 (95.6%)
HPV6/11	28 (6.5%)	6 (1.4%)	8 (1.9%)	386 (90.2%)
HPV6	23 (5.4%)	7 (1.6%)	4 (0.9%)	394 (92.1%)
HPV11	3 (0.7%)	0 (0%)	11 (2.6%)	414 (96.7%)
Other high-risk HPV types				
HPV26	1 (0.2%)	2 (0.5%)	2 (0.5%)	423 (98.8%)
HPV35	2 (0.5%)	1 (0.2%)	3 (0.7%)	422 (98.6%)
HPV39	18 (4.2%)	6 (1.4%)	4 (0.9%)	400 (93.5%)
HPV51	30 (7.0%)	13 (3.0%)	5 (1.2%)	380 (88.8%)
HPV53	17 (4.0%)	13 (3.0%)	8 (1.9%)	390 (91.1%)
HPV56	20 (4.7%)	2 (0.5%)	9 (2.1%)	397 (92.8%)
HPV59	12 (2.8%)	22 (5.1%)	2 (0.5%)	392 (91.6%)
HPV66	19 (4.4%)	27 (6.3%)	0 (0%)	382 (89.3%)
HPV68	3 (0.7%)	5 (1.2%)	9 (2.1%)	411 (96.0%)
HPV70	2 (0.5%)	5 (1.2%)	3 (0.7%)	418 (97.7%)
HPV73	24 (5.6%)	6 (1.4%)	3 (0.7%)	395 (92.3%)
HPV82	2 (0.5%)	8 (1.9%)	0 (0%)	418 (97.7%)

1: Hybrid Capture 2 (HC2) and Linear Array testing performed on pre-vaccination surveillance specimens

2: the in-house Luminex assay was performed on post-vaccination surveillance systems

C2: Bootstrapping methods for comparing pre- and post-vaccination HPV prevalence with estimated sensitivity and specificity

The below is the Stata code which I wrote to calculate an adjusted odds ratio which additionally adjusts for the assay change and the uncertainty in the sensitivity and specificity estimates comparing the two assays. This is an example code for HPV31.

```
* Create sample set for validation study to calculate sensitivity and specificity estimates
* HPV 31
clear
set obs 428
* tp=tru positives; tn=true negatives; fp=false positives; fn=false negatives
local tp=16
local tn=403
local fp=7
local fn=2
local 1=`tp'+`fp'
local 2=`tp'+`fp'+1
local 3=`tp'+`fp'+`fn'
gen hc2=0
gen luminex=0
replace hc2=1 in 1/`1'
replace luminex=1 in 1/`tp'
replace luminex=1 in `2'/`3'
save " validation_hpv31.dta", replace

* Create file for Bootstrapping
use " HPV chlamydia post imms_clean.dta", clear
gen sensitivity_`type'=1
gen specificity_`type'=1
replace sensitivity_31=(16/18) if survey=="pre"
replace specificity_31=(403/410) if survey=="pre"
keep hpv31 survey recruit_venue age chlamydia LA_valid age_group sensitivity_31 ///
/// specificity_31 year
gen postpre=1 if survey=="pre"
replace postpre=2 if survey=="post"
gen postpre2=1 if survey=="pre"
replace postpre2=2 if survey=="post" & (year==2010 | year==2011)
replace postpre2=3 if survey=="post" & (year==2012 | year==2013)
```

```
save " HPV chlamydia post imms_clean_bootstrap.dta", replace

qui {
set seed 832015
forvalues i=1/1000 {
	use "validation_hpv31.dta", clear
	bsample
	diagt luminex hc2
	local sens=r(sens)
	local spec=r(spec)
	use " HPV chlamydia post imms_clean_bootstrap.dta", clear
	gen sens=1
	gen spec=1
	replace sens=(`sens'/100) if survey=="pre"
	replace spec=(`spec'/100) if survey=="pre"
	keep if LA_valid & age_group==1
	bsample
	logitem hpv31 postpre2 recruit_venue age chlamydia, sens(sens) ///
/// spec(spec) iterate(100)
	local or = exp(_b[postpre])
	noi di `sens' "," `spec' "," `or'
}
```

* Use above 2.5th and 97.5th percentile from above outputs as lower and upper confidence intervals

Appendix D: Collection and validation of HPV vaccination records from CHIS

D1: Letter to Cornwall GP re validation study

«GP_Title» «GP_Forename» «GP_Surname»
«Practice_Name»
«Add_Line_1»
«Add_Line_2» «Add_Line_3»
«Add_Line_4» «Add_Line_5»

dd/mm/yyyy

Dear «GP_Title» «GP_Surname»,

Re: Post-vaccination HPV infection in young women in England

Public Health England (PHE) is responsible for conducting surveillance of HPV to enable ongoing evaluation of the HPV Immunisation Programme. As part of this responsibility, PHE have established surveillance of HPV infections in young women, using suitable residual samples. PHE has approval from the National Information Governance Board (NIGB) to conduct this surveillance (individual patient consent is not required).

The Department of Microbiology at Royal Cornwall Hospital have been providing residual samples for this HPV infection surveillance. Before we anonymise and test these samples for HPV, we link the samples with HPV vaccination details held at Public Health England. The resulting anonymised linked data are used to assess the effectiveness of HPV vaccination.

In order to validate the results of this surveillance, we need to verify the accuracy of the data on the women's vaccination status. We are carrying out this verification for a small number of patients in your area. We liaised with the Cornwall LMC and NHS England South (SW) Medical Director who are supportive of this work. I am thus contacting you to request any information you hold in medical records about the HPV vaccination status of the «NUMBER» patients listed on the enclosed form. All we need is information on their vaccination status - please could you complete the enclosed form for your patient(s) and return it in the enclosed pre-paid, self-addressed envelope? If you prefer, you can send it by encrypted email to david.mesher@nhs.net.

All information provided will be treated in strict confidence and held in compliance with PHE policies on data security. This is unlinked anonymous surveillance and therefore after linking these HPV vaccination data, all patient identifiable information (including the NHS number) will be deleted before the HPV testing is performed.

We appreciate the time and effort involved in providing this valuable follow-up information to us. If you have any questions regarding this letter or general enquiries regarding the surveillance then please contact me on the above email address or by telephone (020 8327 6807).

Yours sincerely

David Mesher,
Senior Scientist (HPV Epidemiology)

Alison Mackenzie
Consultant in Public Health Medicine
Screening and Immunisation Lead

In strict medical confidence

NHS number	HPV vaccination status (please tick one box for each patient)									
4000000001	No record of patient	□	Unvaccinated	□	One-dose received	□	Two-doses received	□	Three-doses received	□
4000000002	No record of patient	□	Unvaccinated	□	One-dose received	□	Two-doses received	□	Three-doses received	□
4000000003	No record of patient	□	Unvaccinated	□	One-dose received	□	Two-doses received	□	Three-doses received	□
4000000004	No record of patient	□	Unvaccinated	□	One-dose received	□	Two-doses received	□	Three-doses received	□
4000000005	No record of patient	□	Unvaccinated	□	One-dose received	□	Two-doses received	□	Three-doses received	□

Appendix E: Supplementary Table for Paper 2: Continuing reductions in HPV16/18 in a population with high coverage of bivalent HPV vaccination in England: an ongoing cross-sectional study (Section 6.2)

Supplementary Table 1: Pre- and post-immunisation prevalence of nonavalent HPV types among women with a non-vaccine high-risk HPV type, by age

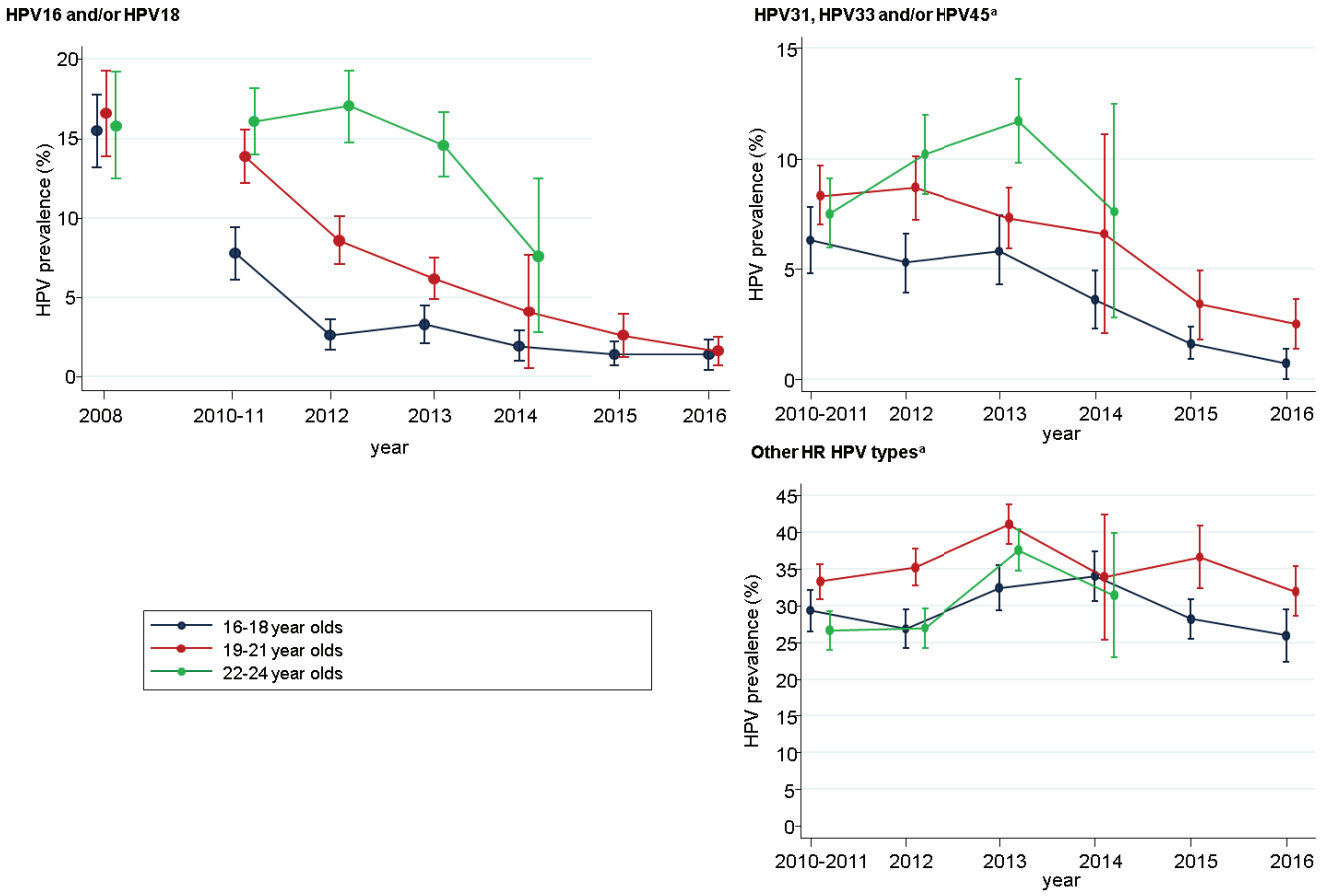
HPV type	Pre-vaccination prevalence (%) 2008 (95% CI) n=610	Post-vaccination prevalence (%) 2010-2011 (95% CI) n=1277	Post-vaccination prevalence (%) 2012-2013 (95% CI) n=1332	p-value for trend
16-18 years				
[Estimated HPV16/18 vaccination coverage]	[0%]	[60.2%]	[73.4%]	
Nonavalent HPV types[1]				
HPV31/HPV33/HPV45/HPV52/HPV58	58.2 (52.2 - 64.3)	51.7 (46.2 - 57.2)	44.8 (39.5 - 50.0)	0.001
HPV31/HPV33/HPV45	33.7 (27.9 - 39.5)	20.1 (15.6 - 24.5)	17.6 (13.6 - 21.6)	<0.001
HPV31	14.9 (10.6 - 19.3)	1.6 (0.2 - 2.9)	3.7 (1.7 - 5.7)	<0.001
HPV33	9.6 (6.0 - 13.2)	10.3 (7.0 - 13.7)	7.9 (5.1 - 10.8)	0.444
HPV45	11.5 (7.6 - 15.4)	8.5 (5.4 - 11.5)	6.5 (3.9 - 9.1)	0.031
HPV52	16.1 (11.6 - 20.6)	25.1 (20.3 - 29.9)	19.3 (15.1 - 23.4)	0.478
HPV58	14.9 (10.6 - 19.3)	11.6 (8.1 - 15.1)	11.6 (8.3 - 15.0)	0.239
19-21 years				
[Estimated HPV16/18 vaccination coverage]	[0%]	[21.4%]	[41.1%]	
Nonavalent HPV types[1]				
HPV31/HPV33/HPV45/HPV52/HPV58	56.5 (49.8 - 63.1)	54.2 (50.1 - 58.3)	50.4 (46.1 - 54.7)	0.096
HPV31/HPV33/HPV45	31.0 (24.8 - 37.2)	22.2 (18.8 - 25.6)	21.9 (18.3 - 25.4)	0.019
HPV31	17.6 (12.5 - 22.7)	5.8 (3.9 - 7.7)	6.7 (4.5 - 8.8)	<0.001
HPV33	7.4 (3.9 - 10.9)	7.3 (5.2 - 9.5)	8.6 (6.2 - 11.0)	0.498

HPV45	9.7 (5.7 - 13.7)	9.4 (7.0 - 11.8)	8.0 (5.7 - 10.3)	0.371
HPV52	15.3 (10.4 - 20.1)	25.7 (22.1 - 29.3)	25.7 (21.9 - 29.4)	0.016
HPV58	18.5 (13.3 - 23.7)	11.9 (9.2 - 14.5)	9.9 (7.3 - 12.4)	0.002
22-24 years				
[Estimated HPV16/18 vaccination coverage]	[0%]	[0%]	[1.1%]	
Nonavalent HPV types[1]				
HPV31/HPV33/HPV45/HPV52/HPV58	63.2 (54.9 - 71.5)	57.5 (52.6 - 62.5)	62.7 (58.2 - 67.2)	0.571
HPV31/HPV33/HPV45	33.8 (25.7 - 42.0)	24.6 (20.3 - 28.9)	30.0 (25.8 - 34.3)	0.989
HPV31	12.0 (6.4 - 17.6)	7.8 (5.1 - 10.5)	8.2 (5.6 - 10.7)	0.296
HPV33	9.8 (4.7 - 14.9)	6.5 (4.0 - 8.9)	10.4 (7.6 - 13.2)	0.326
HPV45	15.8 (9.5 - 22.1)	11.4 (8.2 - 14.6)	12.4 (9.3 - 15.4)	0.498
HPV52	19.5 (12.7 - 26.4)	26.9 (22.5 - 31.4)	28.7 (24.5 - 32.9)	0.065
HPV58	11.3 (5.8 - 16.7)	9.8 (6.9 - 12.8)	10.2 (7.4 - 12.9)	0.809

1: defined as the additional HPV types included in the nonavalent vaccine (31, 33, 45, 52 and 58)

Appendix F: Changes in HPV prevalence in the post-vaccination period among women negative for chlamydia

Figure F1: Prevalence of HPV infection by year of sample collection (restricted to women who tested negative for chlamydia)



Appendix G: Serological surveillance in sexual health clinics

G1: Laboratory protocol for collection of residual sera at sexual health clinics (formerly known as GUM clinics; example version from June 2014)

Post-immunisation monitoring of HPV seroprevalence in young women attending GUM clinics in England

PROTOCOL FOR SUBMITTING RESIDUAL SERA SAMPLES

1. SELECTING SAMPLES

1.1 Sample Selection

All samples included in this study are from women aged 16-19 years old. We will request residual samples from women who have had a chlamydia, gonorrhoea and syphilis test **OR** a chlamydia, gonorrhoea, syphilis and HIV test.

Enclosed is a list of randomly selected patients with **Patient ID number, clinic ID number/name** and **date of attendance** whose samples we would like you to retrieve and submit. These lists will be sent quarterly for collection and submission. **Please note**: **Identifiers from GUMCAD should be considered patient identifiable information and only accessed by those with relevant permissions.**

The clinic ID number/name and patient ID number must match exactly the identifiers on the list provided. The date of clinic attendance should be within one week of the clinic attendance date provided (if there is a discrepancy between 1-7 days then this should be noted in the space provided on the list).

In addition, samples should be frozen (preferably at below -70^{o}C if possible although this is not essential):

2. SUBMITTING SAMPLES

2.1 Labelling samples

All sample tubes should be labelled with the clinic identifier, unique patient identifier and date of attendance. Please remove any other patient identifiers from the sample tube.

Details of where to send the sample tube and completed lists are given below.

2.2 Sample submission

All serum samples should be frozen in secure, appropriately-labelled packaging including freezer packs or dry ice. Serum vials should be rigid polypropylene with a screw-cap with O-ring seal, with a capacity of no more than 2 mL. Please ensure a minimum volume of ~250µl. **Please ensure that only the clinic name/ID number, unique patient ID number and date of attendance are present on the sample vial.**

Samples should be sent to the following address (labels are provided for your convenience) as frozen samples (details for payment are included below):

Ezra Linley
GUM sera for post-immunisation HPV seroprevalence in young women
PHE Seroepidemiology Unit/ Vaccine Evaluation Unit
Public Health England,
Public Health Laboratory, Manchester,
Manchester Medical Microbiology Partnership
2nd Floor, Clinical Sciences Building 2
Manchester Royal Infirmary, Oxford Road
Manchester, M13 9WL, UK

Ideally samples should be sent at the beginning of the week. Please **only** send samples during 8am to 4pm Monday-Friday (excluding bank holidays). Please include a fax number so that the receiving laboratory can send you a fax to confirm receipt of the samples.

2.3 Data submission

Please complete the relevant section of the lists of eligible samples (complete all white sections). Please mark which samples have been retrieved and submitted and where appropriate indicate why a sample could not be provided, if possible. **Completed lists should be sent with the samples to the above address**. Completed lists should be enclosed in an envelope marked "Private and Confidential" which is enclosed in another plain envelope addressed to the above address.

Please retain a copy of the list locally until you receive confirmation from David Mesher at PHE Colindale that this can be destroyed (i.e. when all samples have been received and eligibility verified). When you have received such confirmation, you must destroy the temporary list as confidential waste.

3. AFTER SAMPLE AND DATA SUBMISSION

3.1 Payment for samples

A sum of £4.00 will be paid for each sample submitted with corresponding data (recorded on the Temporary List). Sites will be paid on a six-monthly basis (from first sample submission). Please send an invoice to David Mesher, Dept HIV/STI, Public Health England, 61 Colindale Avenue, London, NW9 5EQ every six-months for the total number of samples provided in the specified time-period clearly indicating that the invoice is for the collection of GUM sera samples for HPV testing. Any costs incurred for delivery of frozen samples should be itemised separately on the invoice.

3.2 HPV testing and storage

Serum specimens will be tested for specific neutralising antibodies to HPV 16 and 18 using enzyme-linked immunosorbent assay (ELISA). The case definitions will be to assess whether an individual is positive due to vaccination against HPV16/18. It may also be possible to determine whether an individual has received full vaccination (i.e. 3 doses) based on their titre levels.

4. SAMPLE SELECTION

4.1 Target number of samples

A list of **Patient ID number, clinic ID number** and **date of attendance** for selected samples for 2013 are enclosed. There is no need to send any additional samples for these years.

The table below shows the number of samples we are requesting by age, for 2013 and 2014. These numbers are just for your information as we have selected appropriate samples in the enclosed list and 2014 samples will be requested as data becomes available. These numbers are approximate and may vary slightly.

	Target number of samples	
Age	**2013**	***2014***
16y	65	*65*
17y	95	*85*
18y	85	*85*
19y	95	*85*
Total	340	*320*

Public Health England

SHHAPT Code Look-Up

Sexual Health & HIV Activity Property Types - Summary of Definitions (2017 update)

DIAGNOSIS, CONDITION OR DISEASE		SHHAPT
BV & anaerobic balanitis		C6B
Balanitis / vaginitis / vaginosis (other causes)		C6C
Candidosis		C7
Cervical cytology	Minor abnormality	P4A
	Major abnormality	P4B
Chancroid		C1
Chlamydia		C4
Donovanosis		C3
Gonorrhoea		B
Hepatitis	A - acute infection	C15
	B - 1st diagnosis	C13
	C - 1st diagnosis	C14
Herpes (anogenital)	1st episode	C10A
	Recurrent episode	C10B
HIV	Known positive	H
	New diagnosis	H1
	New diagnosis – acute infection	H1A
	New diagnosis – AIDS defined	H1B
	HIV related care	H2
LGV	Lymphogranuloma venereum	C2
Molluscum contagiosum		C12
Mycoplasma genitalium		C16
NSGI (non-specific genital infection)		C4N

DIAGNOSIS, CONDITION OR DISEASE		SHHAPT
Ophthalmia neonatorum		C5B
Other conditions requiring treatment		D2B
Pediculosis pubis		C9
PID & epididymitis		C5A
Pregnant	1-12 weeks	PR1
	13-28 weeks	PR2
	29-40 weeks	PR3
Scabies		C8
Sexual assault	Acute - within 7 days	40
	Non-acute - more than 7 days	41
Shigella	*flexneri*	SG1
	sonnei	SG2
	Other / unspecified	SG3
Syphilis	Primary	A1
	Secondary	A2
	Early latent	A3
	Cardiovascular	A4
	Neurosyphilis	A5
	Other late and latent	A6
	Congenital	A7A
Trichomoniasis		C6A
UTI	Urinary tract infection	D2A
Warts (anogenital)	1st episode	C11A
	Recurrent episode	C11D

SERVICE PROVIDED		SHHAPT
Cervical cytology done		P4
Contraception		P3
Hepatitis A immune		O22
Hepatitis A vaccination	1st dose	O20
	2nd dose	O21
Hepatitis B immune		P2I
Hepatitis B vaccination	1st dose	P2A
	2nd dose	P2B
	3rd dose	P2C
	4th dose	P2D
	Booster	P2E
HPV vaccination	1st dose	W1
	2nd dose	W2
	3rd dose	W3
No service and/or no treatment required		D3
Partner notification	Initiated*	PN
	Chlamydia	PNC
	Gonorrhoea	PNG
	Hepatitis A	O23
	HIV	PNH
	NSGI	PNN
	PID / epididymitis	PNP
	Syphilis	PNS
	Trichomoniasis	PNT

SERVICE PROVIDED			SHHAPT
Patient type	Prisoner		Z
	Sex worker		SW
	Sexual reproductive health		SRH
PEPSE	Post exposure prophylaxis sexual exposure		PEPS
Referral type	from NCSP		REF1
	to GUM (Level 3)*		REF2
	from home testing / sampling		REF3
Testing -	**HIV**	HIV antibody test	P1A
		HIV test offered & declined	P1B
		HIV test not appropriate	P1C
	STIs	Chlamydia only	T1
		Chlamydia & gonorrhoea	T2
		Chlamydia, gonorrhoea & syphliis	T3
		Chlamydia, gonorrhoea, syphilis & HIV	T4
		HSV (herpes simples virus)	T5
		Hepatitis A / B / C	T6
		Syphilis & HIV	T7
		Self sampling	T8
		STI tests not required	T9
		Rapid testing	T10
		Microscopy	TS
		3 site testing	TT

SERVICE PROVIDED – PrEP IMPACT		SHHAPT
PrEP eligibility	Criterion 1: MSM / trans woman	O31
	Criterion 2: HIV+ partner	O32
	Criterion 3: Others at risk	O33
PrEP prescription	30 tablets	O51
	60 tablets	O52
	90 tablets	O53

SERVICE PROVIDED – PrEP IMPACT		SHHAPT
PrEP offer & use	Starting or continuing DAILY regimen	O41
	Starting or continuing EVENT based regimen	O42
	Continued through other source	O43
	Offered & declined	O44
	Stopped	O45
Patient characteristic	Transgender	O60

SUFFIX NAME	VALID SHHAPT CODE	SUFFIX
Diagnosed previously elsewhere	A1, A2, A3, A4, A5, A6 A7A, B, C4, C6A, H1, H1A, H1B	X
Medication given	B, C4, C10A, C10B, C11A, C11D	M

SUFFIX NAME	VALID SHHAPT CODE	SUFFIX
Pharyngeal infection	B, C2, C4	O
Quadrivalent HPV vaccine	W1, W2, W3	Q
Rectal infection	B, C2, C4, C4N	R

*For use in Level 2 (non-GUM) services, optional in Level 3 (GUM) services